THE RÔLE OF VITAMIN A IN THE NUTRITION OF CALVES*

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INTRODUCTION

Vitamin A has been found to be an indispensable factor in the diet of a number of species of animals. The most characteristic symptoms of a deficiency resulting from its absence, determined especially in case of small laboratory animals, are failure to grow, xerophthalmia, respiratory trouble, intestinal disturbances, death and, with mature animals, failure of normal reproduction and rearing of young. The question seems pertinent as to how far the results obtained with laboratory animals are applicable to domestic, ruminating animals, concerning which no systematic study has been found in the literature.

It will be the purpose of this paper to set forth the results of an investigation to determine if ruminants, as represented by calves, require a supply of vitamin A for normal growth and well-being. Although the problem was attacked largely from the scientific aspect, the economic consideration was kept in mind. With approximately 4,500,000 calves of the dairy breeds alone raised yearly any investigation dealing with their normal nutrition may be of tremendous practical significance.

HISTORICAL REVIEW

The discovery of vitamin A was based upon the fact that it is present in butterfat, but not in lard (1) (2). The vitamin was

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designated as "fat-soluble A" by McCollum and Kennedy (3) because of its solubility in fats and fat solvents. However, the vitamin is not necessarily associated with fats as, for example, when found in green plants. Also recent investigations (4) (5) have shown that there are two fat-soluble vitamins, one specific in the prevention of xerophthalmia, the other in the prevention of rickets. The anti-xerophthalmic factor is now usually designated as vitamin A and the anti-rachitic factor as vitamin D.

The chemical nature of vitamin A is unknown. The vitamin is quite stable towards heat in the absence of air and oxidizing agents. The body has a considerable capacity for storage of vitamin A, especially in the liver (6).

Some of the symptoms of vitamin A deficiency in the rat are failure to grow (1) (2), xerophthalmia (7) (8), infections of the respiratory tract (9) (10), intestinal atrophy and thrombopenia (11), and osteoporosis (12). Cessation of growth, keratomalacia and an epizootic were observed in guinea pigs on a vitamin A deficient diet (12) (13). Rabbits ceased to grow and developed xerophthalmia on such a diet (14). Chickens likewise ceased to grow (15) and developed xerophthalmia (16) (17) on a diet lacking vitamin A.

Kittens fed on a vitamin A deficient diet ceased growing, developed diarrhea and showed intestinal atrophy (18). Dogs on such a diet developed xerophthalmia (19). Pigs would not grow in the absence of vitamin A (20).

In case of the human, especially children, xerophthalmia and keratomalacia are very common when the vitamin A content of the diet is inadequate (21) (22). Failure to grow and respiratory infections are other symptoms.

As yet there is nothing definite in the literature as to the mode of action of vitamin A. Cramer (11) believes that it acts as a hormone stimulating food absorption. Keith and Mitchell (23) think that it is "involved in the metabolism incident to the increased activity of the voluntary muscles." Sherman (24) suggests that vitamin A may be related to tissue building, it possibly being an important constituent of tissue.

EXPERIMENTAL

Factors involved in the problem

The investigator conducting experiments with large animals involving deficient rations is confronted with many difficulties that the worker with small laboratory animals does not meet. This is especially the case in vitamin deficiency studies. The main difficulty is the formulation of suitable rations. The preparation of a ration of purified food stuffs for large animal experiments is a practical impossibility due to the large amount of food consumed, the length of the growth cycle and the cost of such a ration. The problem then is to select natural foodstuffs that are lacking or deficient in the factor studied or which can be made so in some practical manner. In the case of herbivora the ration must be selected from the limited supply of feeds that this group will consume. Also the ration of herbivora is composed largely of roughage, and it is exceedingly difficult to grow or maintain an animal of this group on a ration composed exclusively of concentrates. Natural roughages consist largely of the leafy part of plants, and as vitamin A is synthesized in the leaves of plants it is a difficult matter to find a natural roughage deficient in the vitamin.

It is a recognized fact that vitamin A is stored in the bodies of the young to a greater or less extent, depending on the diet of the mother. This being the case, it is essential that the ration of the dam be known for the period while the young were *in utero* if an attempt is to be made to measure the requirement of the young for this factor. This is especially difficult in the case of herbivora because of the lack of definite information concerning the vitamin content of many of the ordinary feeds.

PLAN OF EXPERIMENT

The plan was to place a small number of calves on a ration selected as suitable for animals of this kind and thought to be adequate except in vitamin A. At the same time tests of each ingredient of the ration for vitamin A were to be started with rats. With the information at hand from these preliminary tests it was

planned to formulate definite plans for the rations to be used in the more extensive experiments to follow.

Calves were selected so far as possible from dams whose rations were known for the periods while the calves were *in utero* in order to indicate their possible vitamin A storage. The calves were obtained as soon after birth as possible so that their ration was regulated and accurate records of feed consumption and growth kept from an early age. The calves were to be continued on experiment until at least one year of age, unless they developed symptoms of a deficiency before that time, in which case they would either be continued on the same ration until other symptoms developed, or would be given a supply of vitamin A in an attempt to bring them back to normal.

ANIMALS USED

Six experimental animals (E-38, E-47, E-57, E-63, E-64 and E-66) and three control animals (E-46, E-56, and E-65) were used in the investigation. E-38, E-46 and E-47 were placed on experiment before definite information was at hand concerning the vitamin A content of the various ingredients of the ration. All the animals used were either grade or purebred Holsteins.

RATIONS USED

Milk was used as one of the basal constituents of the ration because of the well recognized fact that milk contains the dietary constituents necessary for growing mammals. The calves were given untreated whole milk for a limited period and then changed to whole or skimmilk treated in a manner thought to destroy any vitamin A present as a ration deficient in this factor was desired. Later the calves were given skimmilk remade from oxidized skimmilk powder or, if they would consume it, skimmilk powder in the dry form. The method used in treating the liquid milk and the skimmilk powder was based on the fact that oxidation destroys vitamin A. After some experimenting with calf E-38 the following procedure was adopted for treating the milk: (*a*) 10 cc. of hydrogen peroxide were added for each pound of milk;

(*b*) the milk was heated to 60°C. in a water bath and held at that temperature for one hour; (*c*) liquid oxygen from a storage drum was allowed to bubble through the milk at the rate of about 60 cubic feet per hour for each forty pounds of milk. Skimmilk treated in the above manner was tested with rats to determine its

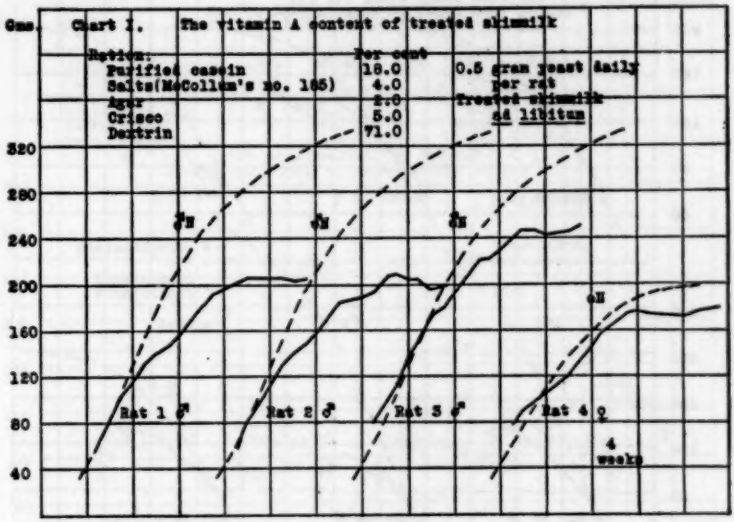

FIG. 1

Rats 1 and 3 were 34 days, and rats 2 and 4, 33 days of age when placed on the test ration. The consumption of treated skimmilk varied from 15 to 35 cc. daily per rat, with an average consumption throughout the test of about 25 cc. The mothers of these rats received a diet very rich in vitamin A as it included 5 per cent alfalfa, 3 per cent cod liver oil and whole milk *ad libitum*. This undoubtedly accounts for the long period of growth before retardation set in. The results indicate a deficiency of vitamin A in the treated skimmilk.

vitamin A content with the results shown in figure 1. The results indicate a deficiency of vitamin A in the skimmilk.

The skimmilk powder used was manufactured by the spray process. The powder was exposed to air and light on the assumption that any vitamin A present would be destroyed by oxidation. The procedure was to spread the powder in shallow layers in pans and boxes placed outside in the sun during the summer

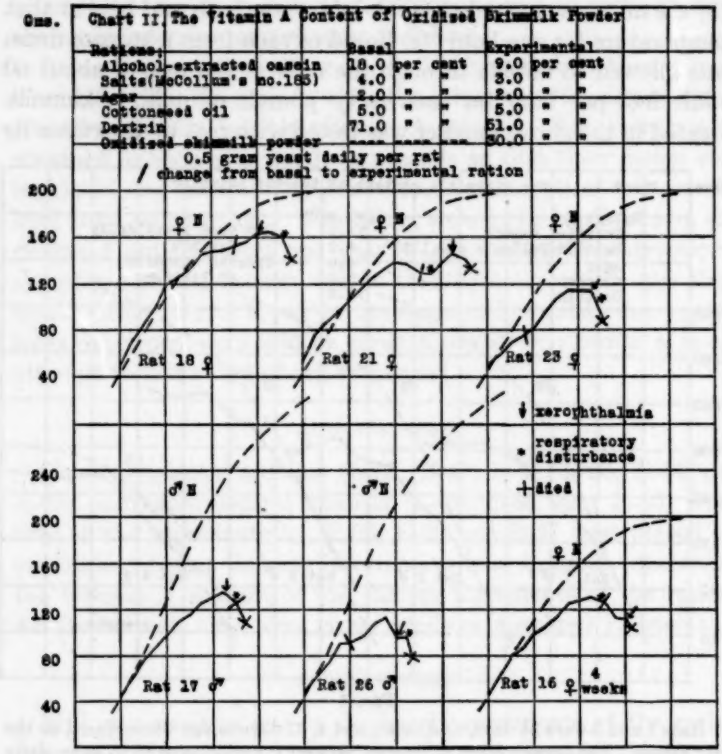

FIG. 2

The rats used in the oxidized skimmilk powder test represented animals from three different litters, the other rats being used in the white corn, wheat straw and dried beet pulp tests. Rats 16, 17, 18 and 21 were placed on the basal ration at 26 days and rats 23 and 26 at 30 days of age. Only in case of rat 17 did xerophthalmia develop before the change to the experimental ration. However, all the other rats later developed xerophthalmia and died of vitamin A deficiency showing that the oxidized skimmilk powder was free of vitamin A. The average weekly food consumption per rat was 46 grams and 44 grams for the basal and experimental rations respectively.

months and inside on a warm cement floor during the winter months. The powder was frequently raked over and was exposed until it changed from its original yellowish to a characteris-

tic white color at which time it also became tallowy in flavor. The oxidation process required five or six days exposure in direct sunlight and three to four weeks in indirect light. The oxidized powder was tested on rats with the results shown in figure 2. The rats in this case were placed on a basal diet lacking in vitamin

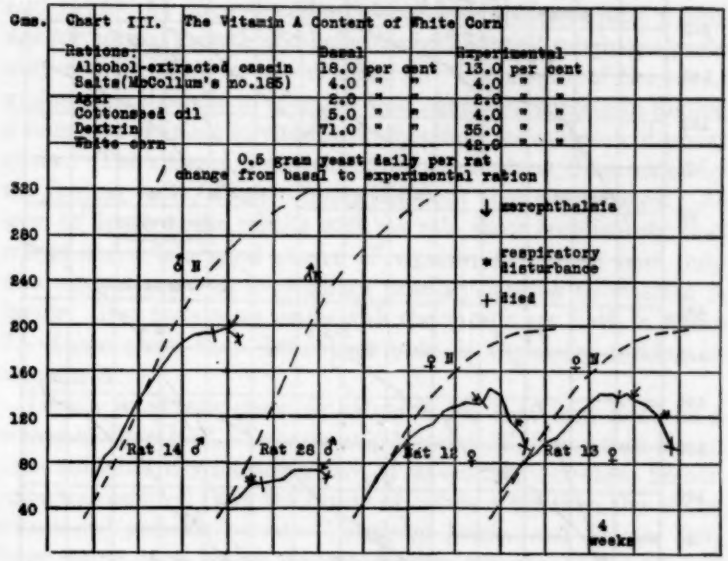

FIG. 3

The four rats used in this test represented three litters. Rat 12 was given the basal ration at the age of 26 days and rats 13, 14 and 28 at 30 days. The change to the experimental ration did not bring about resumption of growth or heal xerophthalmia in the one rat affected. On the other hand, the three other rats developed xerophthalmia before death. The average weekly food intake per rat was about 55 grams of both the basal and experimental rations. The results show that white corn (Rustler White variety) is lacking in vitamin A.

A until they developed symptoms of vitamin A deficiency, at which time the skimmilk powder was substituted for 30 per cent of the diet. The results with six rats show conclusively that the oxidized skimmilk powder was practically free of vitamin A for all of the rats died of vitamin A deficiency.

White corn (Rustler White variety) was used as a source of

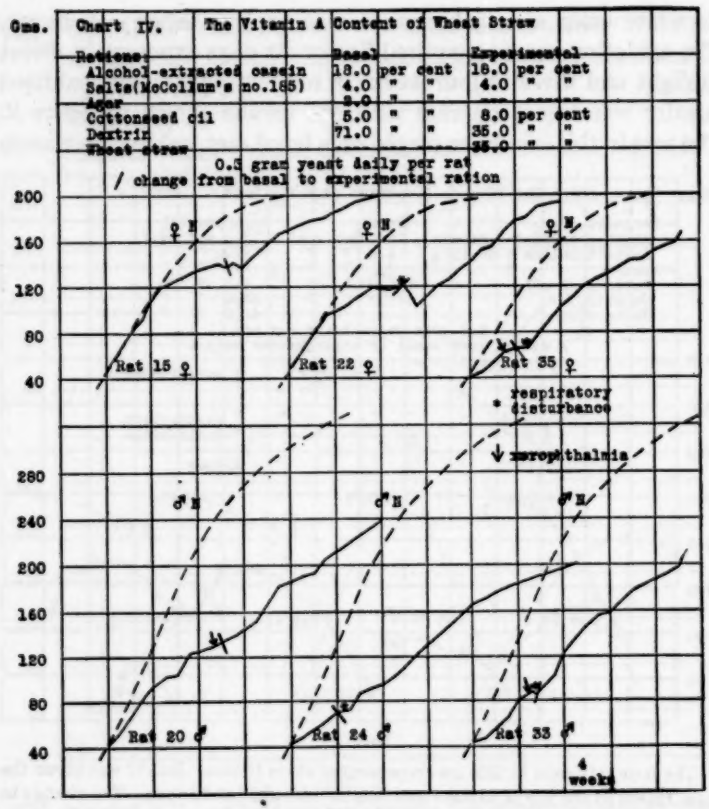

FIG. 4

The 6 rats used in the above test represented four litters. Rats 15, 20 and 22 were placed on the basal ration at 26 days of age, rat 24 at 30 days and rats 33 and 35 at 29 days. The chart shows that a change to the wheat straw ration caused a resumption of growth in case of every rat. The xerophthalmia was healed in five to seven days and the respiratory infection warded off in the rats affected. The average weekly food consumption per rat was 45 grams and 85 grams of the basal and experimental rations, respectively.

energy and vitamin B. Harshaw (25) found that 25 per cent of white corn as the sole source of vitamin B enables rats to make normal growth. Steenbock and Boutwell (26) were unable to demonstrate any vitamin A in white corn. The white corn used

in this experiment was tested with rats to determine its vitamin A content. The results with four rats, as seen in figure 3, show conclusively that white corn (Rustler White variety) is practically free of vitamin A.

Wheat straw was given as a roughage to calves E-38, E-46 and E-47. As pointed out previously, herbivora require roughage for normal growth and behavior and natural roughages probably contain more or less vitamin A. The straws of the cereal grains were thought to offer the best chance of success in finding a roughage deficient in vitamin A; therefore the selection of wheat straw. The vitamin A content of the wheat straw, determined by rat feeding tests, was strikingly different than anticipated. As seen in figure 4, the results with six rats show conclusively that wheat straw is a good source of vitamin A. Dried beet pulp was next selected as a roughage possibly lacking in vitamin A factor. Rat tests were made with the results set forth in figure 7. These show that dried beet pulp is decidedly lacking in vitamin A.

Lemon juice was given to calves E-38, E-46 and E-47 as a source of vitamin C. Osborne and Mendel (27) found that lemon juice contains only a trace, if any, of fat-soluble vitamins. Lemon juice was omitted from the ration of calves other than the above mentioned animals because Thurston, Eckles and Palmer (30) have found that calves do not require vitamin C for normal growth and well-being.

Mineral supplements of calcium carbonate or of calcium carbonate and calcium phosphate were added to the rations of the calves to give an approximate by weight ratio of 1.5 of calcium to 1 of phosphorus which ratio McCollum and co-workers (28) regard as about the optimum for rats.

Cod liver oil was included in the ration of the control animals as a source of vitamin A. In the case of calf E-38 oxidized cod liver oil was given as an anti-rachitic agent during the winter months. The oxidation of the cod liver oil was carried out somewhat in the manner reported by Steenbock and Nelson (5). The other calves were amply supplied with sunshine during most of the experimental periods.

The calves were stabled in individual pens. Shavings were used for bedding. All experimental calves were turned out in a yard together on all days unless the weather was very bad. The yard is located so that it is exposed to the sun the greater part of the day. White corn and wheat straw or dried beet pulp were given to the calves *ad libitum* while the other ingredients of the ration were fed in definite amounts. Salt was always available and water was amply supplied.

Accurate records of feed consumption were kept. Growth was ascertained by weighing every ten days with a three-day weighing period every thirty days and by measuring the height at withers every thirty days. Daily observations of the calves were made and any abnormal symptoms noted. Photographs were taken at various intervals to portray the development of the animals.

RESULTS OF EXPERIMENTS ON CALVES

Experimental calf E-38. Male; dam received pasture seven months and dry feedstuffs two months of gestation period. E-38 was kept on experiment for 402 days without any abnormal symptoms resulting. The growth by weight compared to the Eckles (29) normal Holstein curve is shown in figure 5. As shown in figure 6, the liver of E-38 was found by rat tests to contain considerable vitamin A.

Control calf E-46. Female; dam received dry feedstuffs throughout gestation period. As E-46 was a control animal cod liver oil was fed at the rate of 20 cc. of the oil daily beginning with the 12th day. E-46 was kept on experiment 327 days during which time no abnormal symptoms developed. Her growth curve is shown in figure 5.

Experimental calf E-47. Male; dam received dry feedstuffs throughout gestation period. The ration of E-47 was identical to that of E-46 except that no cod liver oil was given. The growth curve of E-47 is shown in figure 5. This shows that his rate of growth declined rapidly when about 120 days old until at 190 days he was actually losing in weight. At this time he was emaciated in appearance, lacking in vitality and scouring. At 225 days he reacted to the tuberculin test. He continued to decline so that at 230 days he was at the point of death, so cod liver oil was given as a source of vitamin A. Improvement in vitality and in the condition of the fecal matter was soon noticeable

and while he declined further in weight this was soon checked and when taken off experiment at 345 days of age he was gaining at a supernormal rate for that age and was fairly thrifty in appearance. Post-mortem examination showed calcified glandular tuberculosis, chronic peritonitis and chronic pleurisy. Indications were that the calf had been in a more severe state of infection and was gradually recovering.

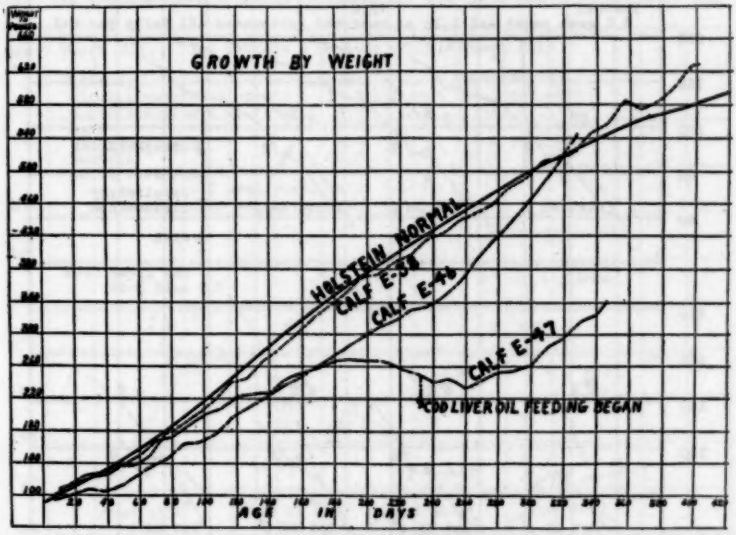

FIG. 5. GROWTH BY WEIGHT OF EXPERIMENTAL CALVES E-38 AND E-47 AND CONTROL CALF E-46

This chart shows that calves E-38 and E-46 were above normal when taken off experiment. Neither of these calves developed symptoms of deficiency throughout the experiment. On the other hand calf E-47 stopped growing at about 180 days and was soon declining in weight. He was near death when cod liver oil feeding began. He slowly recovered and when taken off experiment at the age of 345 days he was growing at a supernormal rate.

Control calf E-56. Male; identical twin with E-57; among other feeds his dam received pasture throughout practically the entire gestation period. Twenty cubic centimeter of cod liver oil was added to the ration daily beginning with the 33rd day. E-56 showed no abnormal symptoms whatever during the 268 days on experiment. His growth curve compared to the normal and to that of E-57 is shown in figure 8.

Experimental calf E-57. Identical twin with control calf E-56.

E-57 received an identical ration to E-56 except that no cod liver oil was given. E-57 was unthrifty and scouring at 75 days of age. The

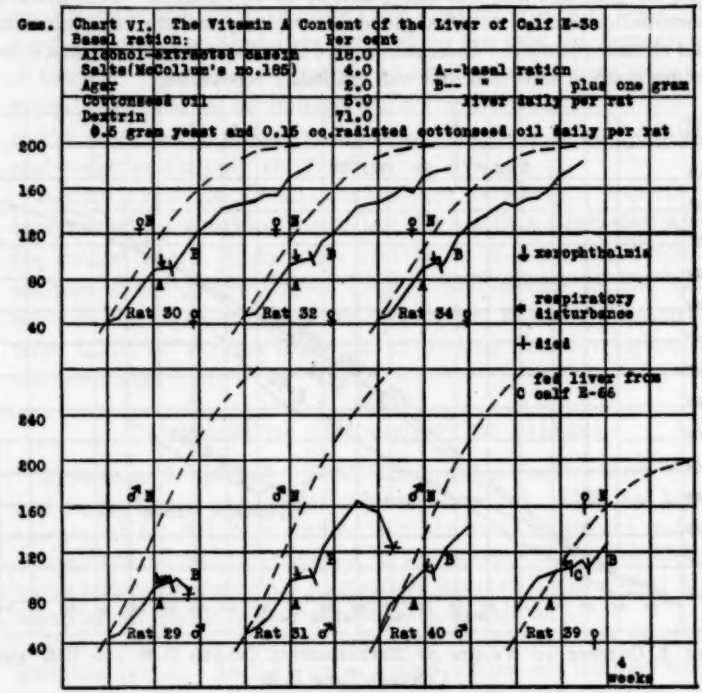

FIG. 6

Rats 29, 30, 31, 32 and 34, littermates, were placed on the basal ration at the age of 29 days. Rats 39 and 40 were started at 31 days of age. One gram of the liver of calf E-38 was given to each rat daily when growth had practically ceased and xerophthalmia had developed. In the case of every rat the xerophthalmia was healed and, except in case of rat 29, growth was resumed. Rat 29 had a bad respiratory infection when the liver supplement was given and did not recover. Rat 31 showed no symptoms of vitamin A deficiency at death. Death was probably due to the fact that a portion of liver that had been kept in a small ice box refrigerator had been fed for a few days. The results show that the liver of calf E-38 contained vitamin A.

scours continued irregularly until at 107 days he was scouring profusely and his eyes were running considerably. His growth curve is given in

figure 8. This shows that he started to decline in rate of growth at about 75 days and continued to decline until his death at the age of 123 days. The results show that E-57 was unable to grow and ward off infections in the absence of vitamin A whereas his twin brother on the control ration grew normally.

Experimental calf E-63. Male; his dam received a ration of alfalfa hay, corn silage and a grain mixture for the first three months of the

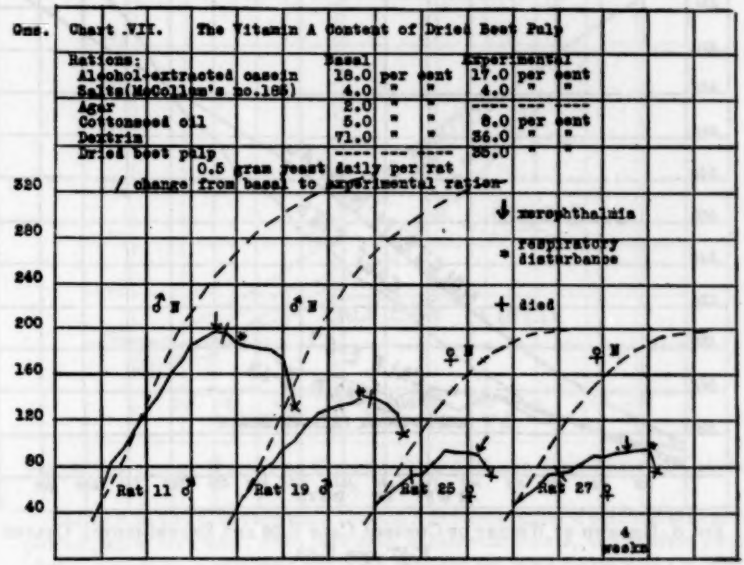

FIG. 7

The four rats used in the dried beet pulp test represented three litters. Rat 11 developed xerophthalmia before the change to the experimental ration and rats 25 and 27 after the change. Rat 19 did not recover from a respiratory disturbance after the change of ration. The average weekly food consumption was 57 grams and 45 grams of the basal and experimental rations, respectively. The results show quite conclusively that dried beet pulp is lacking in vitamin A.

gestation period, then pasture and corn silage for two months, pasture only for the following two months and alfalfa hay, corn silage, and a grain mixture for the two months before calving. Calf E-63 was unthrifty and scouring at two months of age, but as figure 8 shows, he grew at a subnormal rate until about 90 days of age when he was very weak, lacking in vitality and scouring. At the age of 100 days he

went down and would not attempt to arise or stand when put on his feet. His condition did not improve so cod liver oil was added to his ration as a source of vitamin A on the 102nd day. Improvement in the condition of scours, appetite and vitality was almost immediate.

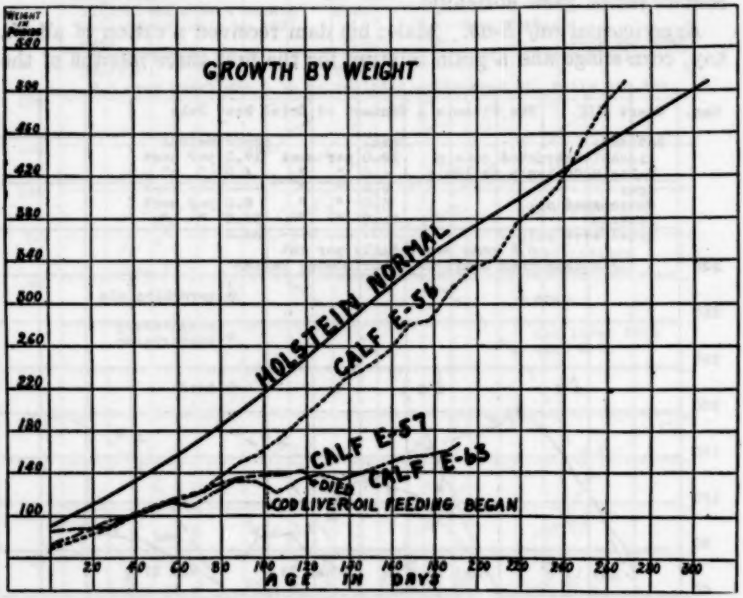

FIG. 8. GROWTH BY WEIGHT OF CONTROL CALF E-56 AND EXPERIMENTAL CALVES E-57 AND E-63

Calves E-56 and E-57 were identical twins. E-56, being a control animal was given 20 cc. of cod liver oil daily in addition to the experimental ration. This calf completed an experimental period of 268 days without showing any abnormal symptoms whatever. On the other hand E-57 died at the age of 130 days showing symptoms of vitamin A deficiency. Calf E-63 received an identical ration to that of calf E-57. At the age of 100 days E-63 was unable to arise and showed symptoms of vitamin A deficiency. Cod liver oil was given as a source of vitamin A and as a result he showed gradual improvement.

He attempted to arise in about ten days, but it was not until the 153rd day that he could get up on all four feet and even then he could not entirely straighten up on his rear legs. As seen in figure 8, E-63 slowly gained in weight after cod liver oil feeding began. The condition of his rear legs did not improve when placed on a normal calf ration.

Postmortem examination showed that the femur of one leg had been broken in two and had partly grown together after the ends had slipped by each other.

Experimental calf E-64. Male; one of a pair of ordinary twins, E-65, a female being the other; his dam received the same ration as the dam

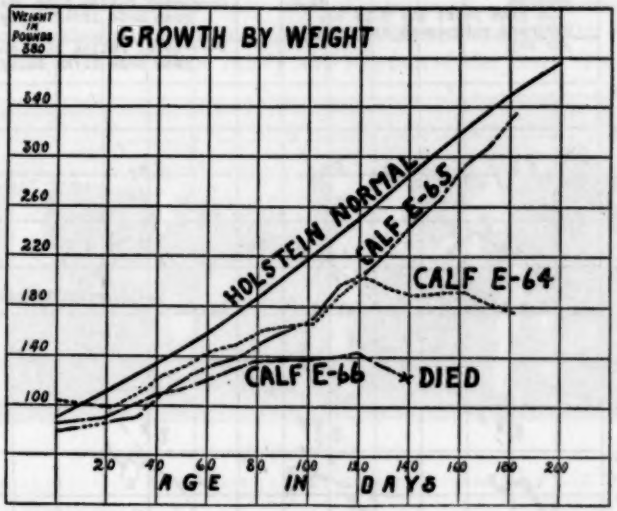

FIG. 9. GROWTH BY WEIGHT OF CONTROL CALF E-65 AND EXPERIMENTAL CALVES E-64 AND E-66.

Calves E-64 and E-66 received a vitamin A deficient ration of oxidized skimmilk powder, white corn meal, dried beet pulp, calcium carbonate, and calcium phosphate. Calf E-65, a twin with calf E-64, received the same ration with the addition of cod liver oil as a source of vitamin A. E-65 showed no abnormal symptoms whatever throughout the experimental period of 183 days. On the other hand calf E-66 died at the age of 139 days and calf E-64 was killed when near death at the age of 183 days. Both of these calves developed xerophthalmia, became blind, ran at the nose, were affected with a chronic condition of scours, and, on post-mortem examination, showed pneumonia, oedema and intestinal atrophy. The results conclusively show that calves require vitamin A in the ration.

of E-63. The growth by weight curve of E-64 is shown in figure 9. At 100 days he did not show the thrifty appearance of his sister on the control ration. At about the 120th day his eyes began to water and strings of mucous were running from his nose. From the 125th day on he declined in weight. At the age of 150 days a puslike discharge

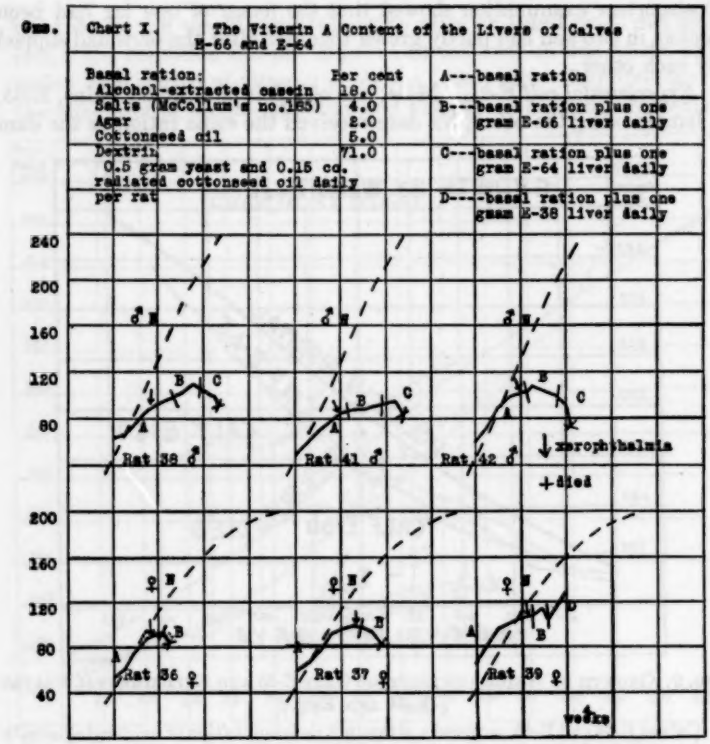

FIG. 10

The six rats used in the above tests were littermates. Rat 40, given E-38 liver, was also from this litter. All of the rats had developed xerophthalmia and practically ceased growing before the liver of calf E-66 was given. This calf died of vitamin A deficiency. In no case did 1 gram of this calf's liver heal xerophthalmia or cause a permanent resumption of growth. Two of the rats died. Of the other four, three were changed to liver from calf E-64, which calf was killed when near death due to vitamin A deficiency, and the fourth, rat 39, was given liver from calf E-38. The rats given E-64 liver did not recover and soon died, whereas rat 39 recovered from xerophthalmia in five days and resumed growth thus corroborating the results shown in figure 6. The results show that vitamin A was practically absent in the livers of calves E-66 and E-64.

was running from his eyes and nose, his feces were very thin and his breathing was interspersed with coughs. Plate 1 shows the marked

contrast in E-64 and his sister at the age of 177 days. At 180 days he was completely blind and xerosis of the eyes was setting in. Long strings of whitish mucous were hanging from his nose. As the calf was in such a miserable state that he would undoubtedly die soon, he was killed on the 183rd day. Growth of the skeleton was totally inhibited during the last twenty days of experiment. Post-mortem examination showed pneumonia, edema of the kidneys, atrophy of the intestines, necrosis of the rumen and sclerosis of the liver. The liver

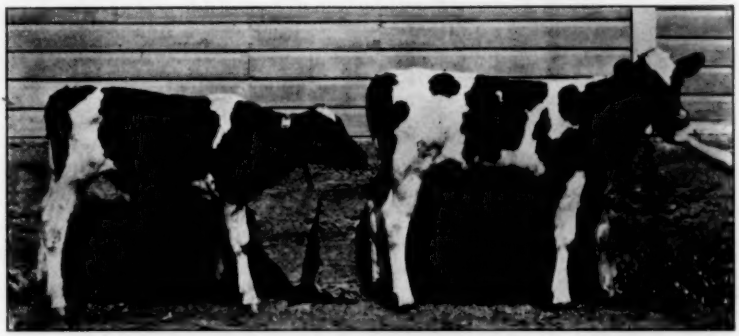

PLATE 1

EXPERIMENTAL CALF E-64 (LEFT IN PICTURE) AND CONTROL CALF E-65 (TWINS)

Age, 177 days.
Weight of E-64, 181 pounds (52 per cent normal).
Weight of E-65, 318 pounds (93.1 per cent normal).
Height of E-64, 90.5 cm. (90.2 per cent normal).
Height of E-65, 97.6 cm. (97 per cent normal).

This picture shows the marked contrast in the size and condition of the twin calves E-64 and E-65 due to the fact that E-64 was on a vitamin A deficient diet, whereas E-65 received the same ration plus cod liver oil as a source of vitamin A. E-64 was totally blind when the above picture was taken, and xerosis of the eyes was starting.

of E-64 was tested for vitamin A storage by feeding to rats with the results shown in figure 10. One gram daily supplements of the fresh liver did not renew growth or heal xerophthalmia in young rats as did the liver of E-38 as shown in figure 6.

Control calf E-65. Freemartin with experimental calf E-64; E-65 was given a ration identical to that of E-64 and E-66 except that she received in addition 20 cc. of cod liver oil daily as a source of vitamin A. E-65 grew at a normal rate and showed no abnormal symptoms through-

out the experimental period. At three months of age there was a marked contrast in the appearance and vitality of E-65 and experimental calves E-63, E-64 and E-66. As shown in figure 9 E-65 had passed the experimental animals in weight at the age of 133 days even though she was considerably the smallest at birth. Plate 1 previously referred to, shows the contrast in E-64 and E-65 at 177 days. E-65 was taken off the experiment on the 183rd day when E-64 was killed.

Experiment calf E-66. Female; her dam received a ration similar to that of the dam of E-63. The growth by weight curve of E-66 is shown in figure 9. She was scouring on the 46th day. She stopped growing in weight at 80 days and 106 days her eyes and nose were starting to run. Her eyes became matterated and she was totally blind on the 139th day. She was very weak and scouring profusely. She died on the 140th day. Post-mortem examination showed pneumonia, nephritis, intestinal atrophy and changes in the rumen. As shown in figure 10, rat feeding tests failed to demonstrate any vitamin A in the liver of E-66.

DISCUSSION OF RESULTS

Vitamin A is shown to be an indispensable factor in the diet of calves. The characteristic symptoms of vitamin A deficiency occur in calves as they do in other species of animals. The practical significance of the results are hard to estimate. The three calves which showed unmistakable symptoms of vitamin A deficiency received rations quite low in vitamin A. Two calves receiving the same ration with cod liver oil added showed no abnormal symptoms whatever. No attempt was made to determine the amount of cod liver oil that would just protect the calves from showing symptoms of a deficiency. However, with the two calves in question, a daily consumption of about 20 cc. of cod liver oil which represented about 0.8 per cent by weight of the ration was sufficient for protection.

One calf consuming wheat straw as 40 per cent of the ration as the only source of vitamin A was able to make normal growth. Another calf on the same ration, but consuming less wheat straw developed symptoms of vitamin A deficiency. However, since the dam was stall-fed, the latter calf undoubtedly had a smaller vitamin A storage at birth than the other calf, whose dam received

pasture for the greater part of the gestation period. The vitamin A storage at birth is probably an important factor in the successful raising of calves.

It is a question whether ordinarily there is much danger of a deficiency approaching disaster in the rations of growing cattle. Calves are usually given whole milk and skimmilk until several months of age, and if they receive a roughage containing vitamin A in addition, there would seem to be an adequate supply of the factor. On the other hand, in areas where whole milk is sold and calves are raised on milk substitutes the chance of a vitamin A deficiency might be considerable. The danger would be augmented if the dams were stall-fed and consuming only dry feedstuffs. This somewhat artificial method of feeding and handling cattle is becoming more common.

While calves probably would seldom show symptoms of vitamin A deficiency such as xerophthalmia and running of the nose, there is every reason to believe that some of the earlier symptoms such as poor growth and a chronic condition of scours might exhibit themselves in the case of some calf rations. In this connection we might refer again to the work of Cramer (11) who believes that vitamins exert a positive action similar to that of hormones, for, when added to the diet of rats that had never suffered from any dietetic deficiency, they produced a stimulating effect on the processes of food adsorption from the intestines and growth. Also Sherman and Campbell (31) have concluded from their work with various proportions of milk in the diet that "evidently there is not only a line to be drawn, but a wide zone to be explored between adequate and optimum nutrition."

In the investigation reported in this paper it was found that calves suffering from vitamin A deficiency improved almost immediately when cod liver oil was given as a source of the vitamin. Especially was this improvement noted in the appetite, in the condition of the fecal matter, in vitality, and in growth. It is possible that many calves showing some of these symptoms might be benefited by the addition of a source of vitamin A, such as cod liver oil, to the ration. A supply of vitamin A approaching the "optimum" of Sherman and Campbell (31) would undoubtedly

aid calves in securing their maximum growth on the food consumed, and in preventing infections.

CONCLUSIONS

1. The experimental results with 9 calves show that vitamin A is an indispensable factor in the diet of calves.

2. The characteristic symptoms of vitamin A deficiency in other species of animals, including failure to grow, xerophthalmia, respiratory troubles, diarrhea and death, occur in herbivora, as represented by calves.

3. Cod liver oil feeding causes a resumption of growth and a disappearance of abnormal symptoms in calves declining on a vitamin A deficient ration.

4. Less than 1 per cent of cod liver oil in a ration otherwise practically free of vitamin A allows calves to grow normally.

5. Vitamin A is present in large amounts in the liver of calves fed normal rations but is absent from the liver of calves fed vitamin A deficient diets.

6. Wheat straw is a good source of vitamin A for ruminants.

7. White corn (Rustler White variety) and dried beet pulp are practically free of vitamin A.

8. Dried beet pulp can be used as a roughage for growing calves provided they receive adequate vitamin A in the other constituents of the ration.

9. Skimmilk powder oxidized by exposure to light and air until it becomes tallowy, is practically free of vitamin A.

REFERENCES

(1) McCollum, E. V., and Davis, M.: Jour. Biol. Chem., 1913, xv, 167.
(2) Osborne, T. B., and Mendel, L. B.: Jour. Biol. Chem., 1913, xv, 311.
(3) McCollum, E. V., and Kennedy, C.: Jour. Biol. Chem., 1916, xxiv, 491.
(4) McCollum, E. V., Simmonds, N., Becker, J. E., and Shipley, P. G.: Jour. Biol. Chem., 1922, liii, 293.
(5) Steenbock, H., and Nelson, E. M.: Jour. Biol. Chem., 1923, lvi, 355.
(6) Sherman, H. C., and Smith, S. L.: The Vitamins. 1922. 273 p. Chemical Catalog Co., Inc., New York.
(7) Osborne, T. B., and Mendel, L. B.: Jour. Biol Chem., 1913, xvi, 423.
(8) McCollum, E. V., and Simmonds, N.: Jour. Biol. Chem., 1917, xxxii, 181.

(9) Daniels, A. L., and Armstrong, M. E.: Jour. Amer. Med. Assoc., 1923, lxxxi, 828.
(10) Mori, S.: Johns Hopkins Hospital Bul., 1922, xxxiii, 357.
(11) Cramer, W.: Lancet, 1923, i, 1046.
(12) Hess, A. F., McCann, G. F., and Pappenheimer, A. M.: Jour. Biol. Chem., 1921, xlvii, 395.
(13) Boock, E., and Trevan, F.: Biochem. Jour., 1922, xvi, 780.
(14) Nelson, V. E., and Lamb, A. R.: Amer. Jour. Physiol., 1920, li, 530.
(15) Mitchell, H. H., Kendall, F. E., and Card, L. E.: Poultry Sci., 1923, ii, 117.
(16) Emmett, A. D., and Peacock, G.: Jour. Biol. Chem., 1923, lvi, 679.
(17) Beach, J. R.: Science, 1923, lviii, 542.
(18) Mackay, H. M. McP.: Biochem. Jour., 1921, xv, 19.
(19) Steenbock, H., Nelson, E. M., and Hart, E. B.: Amer. Jour. Physiol., 1921, lviii, 14.
(20) Zilva, S. S., Golding, J., Drummond, J. C., and Coward, K. H.: Biochem. Jour., 1921, xv, 427.
(21) Blegvad, O.: Amer. Jour. Ophthol., 1924, vii, 89.
(22) Bloch, C. E.: Proc. World's Dairy Congress, 1924, v. i, 447.
(23) Keith, M. H., and Mitchell, H. H.: Amer. Jour. Physiol., 1923, lxv, 128.
(24) Sherman, H. C.: Food Products, 1924, p. 30. The Macmillan Co., New York.
(25) Harshaw, H. M.: Master's Degree Thesis, Univ. of Minn., 1922.
(26) Steenbock, H., and Boutwell, P. W.: Jour. Biol. Chem., 1920, xli, 81.
(27) Osborne, T. B., and Mendel, L. B.: Jour. Biol. Chem., 1920, xlii, 465.
(28) McCollum, E. V., Simmonds, N., Shipley, P. G., and Park, E. A.: Jour. Biol. Chem., 1922, li, 41.
(29) Eckles, C. H.: Mo. Agr. Exp. Sta. Res. Bul. 36, 1920.
(30) Thurston, L. M., Eckles, C. H., and Palmer, L. S.: Journal of Dairy Science, 1926, ix, 37.
(31) Sherman, H. C., and Campbell, H. L.: Jour. Biol. Chem., 1924, lx, 5.

SWEETENED CONDENSED MILK

IV. A REFRACTOMETRIC METHOD FOR DETERMINING TOTAL SOLIDS*

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For many years the various types of refractometers have been extensively used in chemical analysis. These instruments have been so simplified and standardized that they have proved extremely convenient for many purposes.

The determination of the quantity of substance in solution is one of the most important forms of analysis to which refractive principles have been applied, refractive index increasing regularly with the percentage composition of the solution: Estimation of the quantity of alcohol in solution is perhaps the most familiar example. Reagents may be standardized with a fair degree of accuracy by the use of refractometric tables. Since the dry matter in milk serum is quite constant for normal milk, refractive index of the serum furnishes data from which conclusions can be drawn as to whether or not the milk has been watered. The refractometer has found wide employment in estimating the amount of sucrose in solution in sugar house work. The use of this instrument for the determination of dry matter in jams, jellies and marmalades (1) and in honey (2) has recently been recommended.

These are for the most part cases of true solutions. In a product such as condensed milk we have to do partly with true solution, but also with substances in colloidal solution, and, in mere suspension (fat). If, therefore, refraction is to be a meas-

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[1] Work here recorded was done when the senior author was on the staff at Cornell University.

ure of dry matter in such a case, it is necessary that the refractive index of liquids be affected in a regular manner by substances in colloidal solution and in suspension, as well as by those in true solution, Sufficient evidence is at hand for the validity of the hypothesis as applied to colloids: Wintgen (3) demonstrated a regular increase in refractive index with increase in concentration of colloidal As_2S_3, Sb_2S_3, silicic acid, molybdic acid, $Fe(OH)_3$ and tannin; while Reiss (4), Robertson (5) and others have given ample evidence of the possibility of determining the quantity of protein present in colloidal solution by means of refractive index.

Probably no one has ever claimed that a suspended substance, such as fat, might behave similarly, yet there is reason to predict that such would be the case; for it is well known that refractive index depends only on the size and number of molecules in the path of light, and is independent of the state of aggregation. Of course, there must be some limit; the suspended substance must be reasonably well divided. As will later be shown, the fat of condensed milk behaves in the manner predicted.

EXPERIMENTAL

A refractometer of the Abbe type was used. The adjustment was tested from time to time with pure water which should read 1.3330 at 20°C. Readings were always made at 20°, the temperature of the prisms being controlled by circulating water, and it was found best to have the sample at about that temperature before introducing the drops into the instrument.

Samples of condensed milk were obtained from the market, and also through the kindness of the Nestles Food Company, fresh from a vacuum pan in operation in the factory. All samples more than a few hours old were heated to get the lactose into solution; the following experiment shows the necessity for this:

A sample taken from the vacuum pan just at the finish of a batch was observed at intervals for several hours, making refractive index readings and noting the appearance under the

microscope. For the first few hours the refractive index remained constant, even after small crystals of lactose had appeared. However, after standing over night the refractive index had dropped from 1.4742 to 1.4732 at which time the lactose had crystallized to a considerable degree. But upon heating until all crystals had dissolved the refractive index went back to 1.4742.

Total solids were determined in most cases by the A. O. A. C. method, using ignited sea sand for drying residues; in some cases the determination was made on the Mojonnier machine.

TABLE 1

SAMPLE NUMBER	PER CENT TOTAL SOLIDS		REFRACTIVE INDEX AT 20°C.
	(1)	(2)	
1	70.55	70.53	1.4678
2	72.27	72.28	1.4710
3	72.39	72.42	1.4700
4	73.22	73.18	1.4729
5	73.38	73.34	1.4730
6	74.11	74.13	1.4751
7	74.13	74.13	1.4751
8	74.16	74.10	1.4757
9	74.37	74.37	1.4759
10	74.38	74.37	1.4759

CONDENSED MILK WITH 8 PER CENT FAT

As will be shown later, the percentage of fat considerably influences the relation between solids and refractive index, while the other main variable constituent—sugar—has practically no such effect.

Ten samples of 8 per cent milk of various brands were obtained from the market. Probably none was less than a month old. Duplicate determinations of total solids by the A. O. A. C. method, and the refractive indices are recorded in table 1.

Extraordinary care was taken in making determinations both for total solids and refractive index; in many cases two or more operators checked the results.

While other data were accumulated wholly in line with these

results, these alone were used in the calculations, and others will not be given.

By applying the method of the least squares to these data the following formula was derived:

$$T = 70 + 444\ (n - 1.4658)$$

By means of this formula total solids (T) of any sample can be calculated when the refractive index at 20°C. (n) is known.

TABLE 2

For estimating from the refractive index the total solids in condensed whole milk with 8 per cent fat

REFRACTIVE INDEX AT 20°C.	PER CENT TOTAL SOLIDS
1.4600	67.42
1.4610	67.87
1.4620	68.31
1.4630	68.75
1.4640	69.19
1.4650	69.64
1.4660	70.08
1.4670	70.52
1.4680	70.96
1.4690	71.41
1.4700	71.86
1.4710	72.31
1.4720	72.75
1.4730	73.19
1.4740	73.63
1.4750	74.08
1.4760	74.52
1.4770	74.96
1.4780	75.41
1.4790	75.86

Also by the use of this formula a table was prepared by means of which the per cent of total solids may be read off when the refractive index is known (table 2). This should prove more convenient than the formula for regular use.

On several occasions access was had to a condensed milk factory while the condensing pans were in operation. Samples

were taken immediately after striking the batch and in some cases samples were drawn from the pan at intervals before the batch was finished. Total solids were run on a Mojonnier machine in the factory without checking the results. In table 3 will be found the refractive indices at 20°, total solids obtained therefrom applying the table, and also the total solids determined gravimetrically. Considering the fact that the determination of solids in sweetened condensed milk by any gravimetric method is a most difficult operation with great possibility of error, the comparisons obtained here are quite remarkable.

TABLE 3

BATCH	REFRACTIVE INDEX AT 20°C.	PER CENT TOTAL SOLIDS: REFRACTOMETER AND TABLE	PER CENT TOTAL ACIDS: MOJONNIER
A (finish)	1.4749	74.04	74.08
B (1)	1.4674	70.60	70.93
B (finish)	1.4706	72.13	72.18
C (1)	1.4713	72.44	72.80
C (finish)	1.4731	73.23	73.58
D (1)	1.4611	67.91	67.95
D (2)	1.4649	69.60	69.43
D (3)	1.4676	70.78	70.76
D (4)	1.4688	71.31	71.52
D (5)	1.4722	72.84	72.41
D (finish)	1.4735	73.41	73.63

SWEETENED CONDENSED SKIMMILK

As would be expected when a certain percentage of any one constituent is lowered and that of others correspondingly increased, and where the refractive powers of the interchanged constituents are different, then a different solids-refractive index relation would be expected.

A number of samples of skimmilk were procured. The fat content was not determined but it is entirely likely that some was present, and the amount variable. This may account for the slight discrepancy in some of the data. The total solids was determined by the A. O. A. C. method, also the refractive index at 20°C. The results are given in table 4.

TABLE 4

SAMPLE NUMBER	PER CENT TOTAL SOLIDS		REFRACTIVE INDEX AT 20°C.
	(1)	(2)	
1	70.97	71.05	1.4723
2	71.26	71.32	1.4720
3	71.30	71.35	1.4735
4	71.96	71.91	1.4747
5	72.16	72.08	1.4754
6	72.21	72.21	1.4757
7	72.30	72.39	1.4762
8	72.35	72.33	1.4750
9	72.44	72.44	1.4777
10	72.64	72.82	1.4767
11	73.78	73.78	1.4792
12	73.82	73.80	1.4787

TABLE 5

For estimating from the refractive index the total solids in skimmilk

REFRACTIVE INDEX AT 20°C.	PER CENT TOTAL SOLIDS
1.4680	69.29
1.4690	69.69
1.4700	70.08
1.4710	70.47
1.4720	70.86
1.4730	71.26
1.4740	71.65
1.4750	72.04
1.4760	72.43
1.4770	72.83
1.4780	73.22
1.4790	73.61
1.4800	74.00
1.4810	74.40
1.4820	74.79
1.4830	75.19

Upon resolving these results into a formula by the method of the least squares, the following is obtained:

$$T = 70 + 393\ (n - 1.4698)$$

As in the case of whole milk this provides a means for deter-

mining total solids from the refractive index of condensed skimmilk and also for the preparation of table 5.

Since the curves for both whole and skimmilk are almost exactly straight lines the tables can be extended by interpolation without error.

FUNDAMENTAL BASIS FOR THE RELATION BETWEEN TOTAL SOLIDS AND REFRACTIVE INDEX

It seemed desirable to learn whether or not the relationships herein established are based on sound theoretical principles. If it could be so proved then there would be entire justification for claiming that these formulas and tables will hold under all conditions.

Refraction is an additive property. Much data have been presented in the literature to show that the refractive power of a solution is equal to the sum of the refractive powers of its constituents. Of course, any changes in volume occurring when one substance is dissolved in another must be taken into account. This is done by introducing a factor for density. The formula generally used for comparing refractive powers is

$$\frac{n^2 - 1}{(n^2 + 2)d}$$

where n is the refractive index of the substance and d the density. This gives a refractive constant which is independent of the physical state of the substance, and of the temperature. If solute A is dissolved in solvent B then

$$\frac{n^2 - 1}{(n^2 + 2)d} \text{ (of } A) \times \%A + \frac{n^2 - 1}{(n^2 + 2)d} \text{ (of } B) \times \%B = \frac{n^2 - 1}{(n^2 + 2)d}$$

of the solution. This has been found to hold in any number of instances.

By making a complete analysis of condensed milk and obtaining the refractive constant of each constituent, it should be possible to determine whether or not the principle holds in this case.

The refractive constants of some of the milk constituents

can be found in the literature, others cannot: Water is given in many places as 0.20606; this is the figure used in this work. Sucrose is given by Browne (6) as 0.20614. Wiegner (7) obtained 0.1377 for the ash of milk. No value could be found for fat, but by taking the average of a large number of determinations made by others of refractive index and of density of milk fat a refractive constant of 0.2868 was obtained. Robertson (8) gives 1.675 as the refractive index of casein in solution, and 1.39 as the density. These figures give a refractive constant of 0.2703. The refractive index of solutions of albumin of various strengths was obtained from the work of Reiss and Robertson, and the densities from Chick and Martin (9) and from the Chemists' Year Book (Atack); with these figures an average refractive constant of 0.2494 was calculated.

No satisfactory figures could be found for lactose so that the determination was made experimentally: Several solutions of pure lactose were prepared, the refractive index determined to the fifth decimal place by means of a dipping refractometer and density with an accurate picnometer. The refractive constant of anhydrous lactose was found to be 0.20814.

A sample of condensed whole milk and one of skim were analyzed for sucrose, lactose, casein, albumin, fat, ash and moisture. The usual methods of analysis were followed except that albumin and casein had to be calculatd from the total protein since there is no way of separating heat coagulated albumin from casein; after determining total protein, 20 per cent was taken as albumin and 80 per cent as casein. Although the value for moisture was taken as the difference between the sum of the other constituents and 100 per cent, the figures were quite close to those obtained by direct determination.

Table 6 gives for one sample of whole milk the percentage of each constituent, the refractive constant of each, and the value obtained by multiplying these two together. The sum of the last named values is seen to be 0.2165 (a calculated refractive constant for the sample). The refractive constant was determined also from the refractive index of the sample of condensed milk (1.4750), and the density (1.3050), which were found by

experiment. These values give a refractive constant of 0.2158 These results compare favorably, being well within the limits of error, since the refractive constants employed for some of the constituents may be in slight error, and the method of determining albumin and casein is by no means unobjectionable. Also no account is taken of some of the constituents that must be present, such as citric acid.

TABLE 6

CONSTITUENT	PER CENT (P)	REFRACTIVE CONSTANT (K)	$P \times K$
Water	26.56	0.20606	0.054729
Sucrose	45.1	0.20614	0.092969
Lactose (anhydrous)	11.4	0.20814	0.023727
Casein	6.04	0.2703	0.016326
Albumin	1.50	0.2494	0.003741
Fat	8.09	0.2868	0.023202
Ash	1.31	0.1377	0.001803
Sum			0.216497

TABLE 7

CONSTITUENT	PER CENT (P)	REFRACTIVE CONSTANT (K)	$P \times K$
Water	27.82	0.20606	0.057326
Sucrose	45.70	0.20614	0.094206
Lactose	14.30	0.20814	0.029764
Casein	6.67	0.2703	0.018029
Albumin	2.25	0.2494	0.005612
Fat	1.26	0.2868	0.003613
Ash	2.08	0.1377	0.002864
Sum			0.211414

In exactly the same way a sample of condensed skimmilk was carried through. Table 7 gives the results.

Refractive index of this sample was found to be 1.4777 and the density 1.347. This gives 0.2100 for the experimental refractive constant, which again compares favorably with the figure 0.2114 obtained by calculation. All the sources of error mentioned when considering whole milk are likely to be found here also.

This section of the work shows that the refractive power of condensed milk follows the general law of true solutions. It must, therefore, be concluded that the estimation of total solids from refractive index readings is founded on scientific principles.

The fact that the fat contributes its share to the refractive power of the condensed milk in exactly the same way as do the constituents in true and colloidal solution, shows, indeed, that the state of aggregation of the molecules does not influence the additive principle.

It should be pointed out at this point, however, that difficulty may be experienced in applying this theory in many cases. Unless the refractive index of the suspended substance is very near that of the suspending medium there may be such a dispersion of light that the shadow edge in the refractometer is diffused so as to make it impossible to get a satisfactory reading. Fortunately the conditions necessary for obtaining a good reading are fulfilled in sweetened condensed milk. But with evaporated milk, whole milk and ice cream mixes, it is otherwise; it is not possible with any degree of accuracy to read any of these products for the reasons stated. A method such as is here worked out for condensed milk cannot be devised for these other milk products.

As has been pointed out, when lactose crystals are very small, the true refractive index may still be obtained; it is only when the crystals have grown to their usual maximum size that a low reading results. Apparently, therefore, there is a limit beyond which there may be an error due to the large size of the fat globules. Of the many samples of condensed milk examined in this work there was no suspicion of error in any one from this cause; but it is possible that through fat separation, for instance, the globules may become so agglomerated as to cause an incorrect reading.

As has already been suggested, it is important that the percentage of fat in the sample be known within fairly close limits, while a variation in the sugar content is practically without influence. By referring to table 6 it will be seen why this is the case. The refractive constant of sucrose is very close to

that of the condensed milk itself while that of fat is far away. A variation in the standardization for sugar within several per cent would not alter the relation between solids and refractive index as much as a considerably smaller variation in fat. It is recommended, therefore, that if there be 9 per cent, or more, fat, such as is often found in condensed milk manufactured in Europe, a new formula and tables be worked out.

PROCEDURE FOR THE ESTIMATION OF TOTAL SOLIDS IN SWEETENED CONDENSED MILK WITH AN ABBE REFRACTOMETER

If the specimen has been undisturbed for some time, some of the crystallized lactose will have collected at the bottom. In this case, warm by placing the container in hot water, then transfer the entire contents to a mortar and mix well.

Place a few cubic centimeters in a thick walled test tube. Heat by immersing in boiling water until no more lactose crystals are observed under the microscope. During this operation it is important that the test tube be kept stoppered to prevent evaporation, but the stopper may be removed for an instant from time to time to release the pressure. (The needle-like crystals of calcium citrate may remain undissolved without affecting the results.) If the amount taken is not too large, and if the liquid is mixed frequently by inverting the tube, this operation should require five minutes. Cool to about 20°C. by immersion in cold water, then place tubes in a tempering bath held near that temperature for ten minutes.

The temperature of the refractometer should be controlled at 20°C. by circulating water. Examine two or three different drops in the instrument. The different readings should check exactly.

If the sample is an 8 per cent fat product refer to table 2, if skim, use table 5, reading off the total solids from the refractive index. Interpolate where necessary.

Where a specimen is drawn fresh from the vacuum pan, the preliminary treatment is unnecessary. Merely cool the sample to 20° and read.

SUMMARY AND CONCLUSIONS

The successful use of the refractometer in estimating the dry matter in various liquids led to the conclusion that a process might be worked out for sweetened condensed milk.

The refractive indices of a number of samples of condensed milk with 8 per cent fat were determined, and also careful analyses made for total solids, gravimetrically. From these results a formula was derived by means of which the total solids in any sample can be determined from the refractive index. By employing this formula a table was prepared to be used for a similar purpose.

Refractometric readings were made on a large number of samples taken directly from the vacuum pan, and from these figures the total solids was read off on the table. Total solids was also determined on the Mojonnier machine. The comparisons were found to be excellent considering the liability of error which is large in the gravimetric determination of total solids.

From results obtained on a number of samples of condensed skimmilk a formula and table were worked out in the same way and for the same purpose as with whole milk.

The refractive constant of condensed milk is shown to be equal to the sum of the products obtained by multiplying the refractive constant of each constituent by its percentage. This is the fulfillment of a law which holds generally for true and colloidal solutions, and is proof that the solids-refractive index relation applied to condensed milk is scientific and dependable.

The fact that the fat of condensed milk behaves in this respect as do those substances in true and colloidal solution leads to the conclusion that an agglomeration of molecules in masses even beyond the colloidal size influences the refractive index of a liquid exactly as if the molecules were dispersed in true solution. Necessary conditions, however, are that the suspended particles be not too enormous, and that the refractive index of the suspending medium be fairly near that of the suspended substance.

A formula or table giving the relation between refractive index and total solids will hold even if the sucrose content should vary within wide limits, but the fat must not be far from the percentage for which the table and formula are prepared.

Not only should the refractometric method prove useful in estimating total solids in sweetened condensed milk as a matter of laboratory routine, but it may be, and has been, used as a means of determining the finishing point of batches in the factory. Near the finish of the batch samples are drawn from the pan, cooled, and read off on the refractometer with such ease and speed that the proper point of striking is accurately determined.

REFERENCES

(1) CLACHER: International Sugar Jour., 1910, xii, 509.
(2) AUERBACH AND BORRIES: Zeit. Nahr. Genussm., 1924, xlvii, 177; xlviii, 272.
(3) WINTGEN: Kolloidchemische Beihefte, 1915, vii, 251.
(4) REISS: Beit. z. chem. Physiol. u. Path., 1904, iv, 150.
(5) ROBERTSON: Jour. Ind. Eng. Chem., 1909, i, 723; Jour. Phys. Chem., 1909, xiii, 469.
(6) BROWNE: Handbook of Sugar Analysis. Wiley and Sons.
(7) WIEGNER: Milchwirtschaft. Zentr., 1909, v, 473 and 520.
(8) ROBERTSON: The Physical Chemistry of Proteins. Longmans, Green and Co.
(9) CHICK AND MARTIN: Biochem. Jour., 1913, vii, 92.

GENETICS OF BREEDING BETTER DAIRY STOCK*

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Better dairy stock suggests animals meeting the requirements of the breed association to which they may belong, free from physical defects, uniformly high in the production of milk and butter-fat, a conformation pleasing to the eye, and consistent in breeding performance. Genetic research has as one of its objectives the analysis of the laws of inheritance governing these characters. There would seem to be little doubt that such a genetic analysis will furnish the means by which the practical objective, better dairy stock, will be attained, as already we are indebted to the advances in this field for taking the gamble out of and making the results more consistent in breeding better varieties of many things.[1]

The most obvious application of elementary genetics to these problems is that offered by the requirement of the black breeds that animals to be registered in them shall be black in color and nothing else. Spillman (1), Van Damme (2), Wilson (3), Lloyd-Jones and Evvard (4), Gowen (5), Cole and Jones (6) and Templeton (7) have presented evidence to show the dominance of black over red with the segregation of black from red in a three to one ratio in the second generation. Many of the black breeds have originated from crosses of black on red animals, with subsequent selection of the black animals. Under such conditions the red color may lie hidden in the germ cells of the black coated animals for many generations waiting for a mating which shall bring two reds together in the fertilized egg. This egg is then capable of giving rise to a red animal from purebred black parents, an

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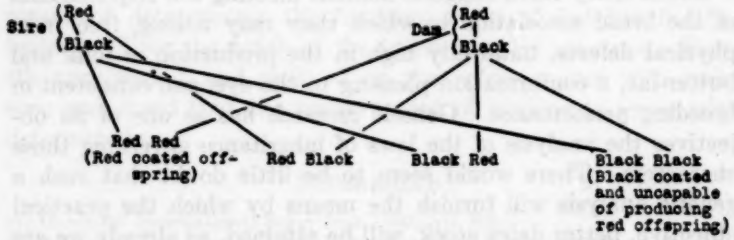
[1] Gowen, John W. 1924. The application of the science of Genetics to the farmers' problems. Sci. Agr., i, 1–12.

animal which, however much it may be worth, cannot be registered. Cases of this sort are not only disappointments but serious economic losses. Our knowledge of genetics shows that both black parents are equally responsible for the production of the red animal. The parents' germ cells, sperms or eggs, are in fact half of them carrying the red producing substance, thus



The damage resulting to a herd from such a mating is difficult to remedy as the production and ultimate loss of the red calf is but the least of it. On the average one out of two of the offspring of such a black sire carrying red also carries the red factor. Such animals on being properly mated are also capable of having red offspring. An animal of this sort is consequently a menace to a herd. In view of this fact it is an open question if the bulls purchased or sold for breeding within the black breeds should not be tested to determine first whether or not these bulls are truly black in their breeding. Our present-day knowledge of inheritance shows that such a test may best be made by breeding the black bull to red cows—if any of the offspring are red the bull should be discarded as he would be known to carry the red factor.

Several of the physical defects found in cattle are now known to be or there is good presumptive evidence that they are due to inherited factors. Such physical defects are congenital cataract (8), defective hair and teeth (9), notched ears (10), abnormal number of toes (11). The inheritance of these defects appears to be of the simple Mendelian type, the elimination of them from the breeding strain being easily possible with the knowledge of how they are inherited.

INHERITANCE OF MILK PRODUCTION

The now clearly recognized differences which exist between the production of some of the breeds, all point to the conclusion that milk production is innate and hereditarily determined between certain breeds. Such reasoning has led certain dairymen to make crosses between breeds in the attempt to combine the high milk production of one breed with high butter-fat percentage of another. It is now known that such crosses are based on false reasoning for while the first generation offspring will generally show higher production than the lower producing breed, it will not show any higher production than the high producing breeds. Furthermore, while the butter-fat percentage of the first generation offspring will be higher than that of the low test breeds it is, in general, considerably lower than that of the high test breeds. These results lead to a total butter-fat production which is about equal or slightly more than the butter-fat yield of the two crossed breeds. Furthermore than this, we know that such crosses lead to second generation offspring which are extremely variable in production and in type. In other words, the dairyman has before him the prospect of years of selection before a stable type is reached with but doubtful results at the end as far as his object of combining high milk production with high butter-fat percentage is concerned. What the dairyman is interested in, therefore, is something more than crosses between breeds. He wants to know whether the differences observed in milk production between different individuals of the same breed are inherited. Should this be true, he may start with a fairly stable type and have to select and breed for but relatively few characters, thus making for a greatly increased chance of success.

The general observation that milk yield differs between strains has indicated that at its foundation many of these differences in producing ability are also hereditary. But how and what are the limitations? For plainly this is not a simple matter, for if it were none of our cows would produce less than 10,000 or 12,000 pounds of milk. The first step to be taken toward the improvement of dairy stock by breeding is then to find out the way in which the

variations or differences in producing ability behave in their inheritance.

The problem[2] of breeding better dairy stock is not so much to produce animals of higher production than those which we have today as it is to be able to reproduce the best of our present-day stock uniformly and at will. In other words, we wish to know what are the significant facts to be taken into consideration in breeding such stock and what items are of but trivial importance. This problem is made doubly difficult by the fact that it is impossible to measure the character, milk production or butter-fat percentage, in the sire. It is, in fact, only possible to arrive

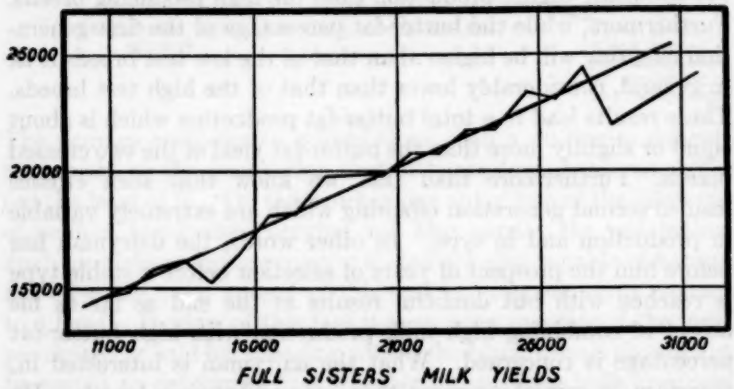


FIG. 1. RELATION BETWEEN THE MILK PRODUCTION OF FULL SISTERS ON A MATURE FORM BASIS FOR THE HOLSTEIN-FRIESIAN ADVANCED REGISTRY COWS

at a measure of the influence of the sire on the production of his offspring by indirect means.

Full sisters have a common sire and common dam. Half sisters have either the sire or the dam in common. If the sire alone determines the production of his daughters the above facts would lead to the expectation that full sisters would resemble each other in their production; that half sisters with a common sire would resemble each other in production and to the same degree that full sisters are correlated; that half sisters with a common dam

[2] For a more complete discussion of this important problem see Milk Secretion, The Williams & Wilkins Company, Baltimore.

would not resemble each other. If on the other hand, both the sire and dam determine the production of their daughters, the full sisters' production would be fairly closely correlated; the half sisters' productions correlated but to a less degree than the full sisters. The correlation between half sisters should be of the same order of strength whether these half sisters had a common sire or dam. If, as the last alternative, the dam determined the production of her daughters with the sire having no influence on production, the expected relations would be, full sisters' production correlated; half sisters' productions where the sire was the common parent not correlated; half sisters with a common dam correlated in their production to the same degree as the full sisters. The actual results derived from a comparison of the production of full and half sisters for Holstein-Friesian cattle are shown in table 1.

TABLE 1

Correlation coefficients for the relation of the milk yields and butter-fat percentages of sisters, Holstein-Friesian Advanced Registry

	MILK YIELD	BUTTER-FAT PERCENTAGE
Full sisters	0.55±0.03	0.46±0.03
Half sisters (common sire)	0.36±0.02	0.37±0.02
Half sisters (common dam)	0.38±0.03	0.22±0.04

The full sisters resemble each other more closely in their milk yields or butter-fat percentages than the half sisters. The half sisters with a common sire or dam appear to resemble each other to about the same degree. The experimental facts thus accord with the hypothesis that the sire and dam are both responsible for the milk yields or butter-fat percentages of their daughters. This conclusion is also supported by similar evidence from Guernsey cattle. The stringency of selection for animals entering the Guernsey Advanced Registry appears to have been greater than it was for those animals entering the Holstein-Friesian Advanced Registry. This selection is known to affect the correlation coefficient. The correlation coefficients for the Guernsey are consequently not directly comparable with those of the Holstein-Friesian breed but should in all probability be somewhat lower.

This differential effect should not materially influence the relative results between full sisters and half sisters. The correlation coefficients describing these relations are given in table 2.

Although the correlation coefficients are lower for the Guernsey Advanced Registry cattle the full sisters stand in the same relation to the half sisters as that displayed in table 1. The full sisters resemble each other more closely in their milk yields and butter-fat percentages than the half sisters. The half sisters, on the other hand, have practically the same degree of resemblance. Thus, here again, there is agreement between experimental facts and the hypothesis that the sire and dam are both responsible for the milk yields and butter-fat percentages of their daughters.

This conclusion is also borne out by such critical results

TABLE 2

Correlation coefficients for the relation of the milk yields and butter-fat percentages of sisters, Guernsey Advanced Registry cattle

	MILK YIELD	BUTTER-FAT PERCENTAGE
Full sisters	0.41±.02	0.44±.02
Half sisters (common sire)	0.13±.02	0.17±.01
Half sisters (common dam)	0.15±.02	0.19±.01

as those obtained from the wide crosses of animals of the high milking breeds with those of the low milking breeds, or of animals of the high butter-fat test with those of the low butter-fat tests. The results appear to be comparable, either way the cross is made, thus indicating an equal effect of the sire and dam on the production of the offspring.

The relation which exists between the milk production or butter-fat percentage of full sisters as contrasted with that of half sisters shows that from a practical breeding standpoint, and as a means of guiding breeding operations, the milk yield or butter-fat percentage of one daughter should be considered very carefully in determining whether or not it is desirable to repeat this mating as such a repetition will tend to beget daughters of similar production. If the daughter is an undesirable cow from a production standpoint, it would appear as if it would be better

to change either the sire or dam, as by so doing the daughter's production would probably be increased.

The influence of the dam on the milk production of her daughters may be measured directly by comparing the productions of the daughters with those of the dams. On making this comparison for Holstein-Friesian cattle, a correlation is found between the production of daughter and dam in milk yield or in

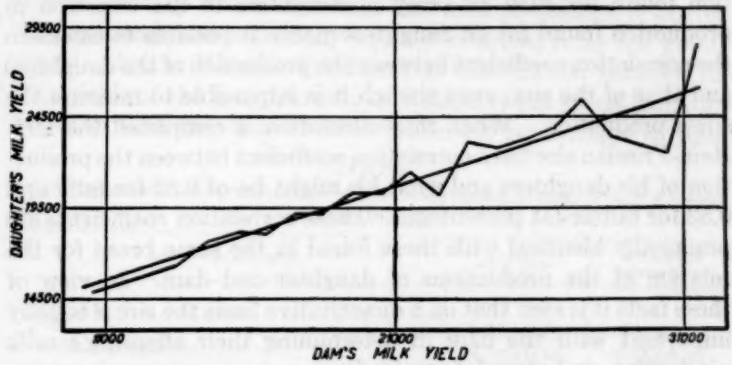


FIG. 2. RELATION BETWEEN THE MATURE FORM PRODUCTION OF DAUGHTERS AND THEIR DAMS FOR HOLSTEIN-FRIESIAN ADVANCED REGISTRY CATTLE

TABLE 3

Correlation coefficients measuring the relation of daughters' and dams' milk yields and butter-fat percentages, Holstein-Friesian and Guernsey Advanced Registries

BREED	MILK YIELD	BUTTER-FAT PERCENTAGE
Holstein-Friesian	0.50±0.02	0.41±0.02
Guernsey	0.36±0.02	0.42±0.02

butter-fat percentage. A similar, although lower, correlation is also found to exist between the productions of daughters and dams as found in the Guernsey Advanced Registry. These correlations are shown in table 3.

Table 3 shows that there is a fairly close relation between the milk productions and butter-fat percentages of daughter and dam in either the Holstein-Friesian or Guernsey Advanced Registry cattle. This relationship is slightly less than that found between

full sisters and more than that found for half sisters. The production record of a dam is consequently of considerable significance in breeding and as a guide to breeding operations.

The amount of influence that the sire exerts on the production of his daughters may be determined from the fact that for any given sire, his daughters must lie within one array or column of the correlation table. The relation of the variation in production found for such an array of daughters to the variation in production found for all daughters makes it possible to calculate the correlation coefficient between the production of the daughters and that of the sire, even though it is impossible to measure the sire's production. When this calculation is completed the Holstein-Friesian sire has a correlation coefficient between the production of his daughters and what his might be of 0.52 for milk and 0.53 for butter-fat percentage. These correlation coefficients are practically identical with those found in the same breed for the relation of the productions of daughter and dam. In view of these facts it is seen that on a quantitative basis the sire is equally important with the dam in determining their offspring's milk production or butter-fat percentage.

The influence of the ancestors further removed is of particular importance in view of the frequent difficulty of obtaining records on animals of the first generation. For the sire these records must be those of the progeny, for the dam the records may be either those of the progeny or her own records for production. Progeny records are of course extremely difficult to obtain unless the parents are quite old.

The interrelation between the production of the granddams, maternal and paternal, with the production of their granddaughters is shown in table 4.

The data of table 4 show that there is a much closer relation between the production of the daughter and dam as contrasted with the relation which is found between the daughter and granddam whether the granddam is on the maternal or paternal side. This conclusion is supported by both the Guernsey and the Holstein-Friesian data. It is true for both milk yield and butter-fat percentage. The grandparents have about an equal relation

between their milk yields and those of their granddaughters or between their butter-fat percentages and those of their granddaughters. In view of these facts it may be concluded that as a basis of constructive breeding, the dams' records are at least twice as important as the granddams' records and that the granddams' records are of about equal importance in indicating the probable production of the daughter and granddaughter. In the Guernsey data it is obvious that milk production or butter-fat percentage are practically equally inherited from the grandparent. In the Holstein-Friesian data the relation between the milk yields of the grandparents with those of the granddaughters is greater than that found for the butter-fat percentages of these

TABLE 4

Relation of parents' and grandparents' performance records to those of their daughters and granddaughters

BREED AND ANCESTOR	MILK YIELD	BUTTER-FAT PERCENTAGE
Guernsey:		
Dam	0.36±0.02	0.42±0.02
Paternal granddam	0.16±0.01	0.15±0.01
Maternal granddam	0.20±0.02	0.20±0.02
Holstein-Friesian:		
Dam	0.50±0.02	0.41±0.02
Paternal granddam	0.26±0.04	0.09±0.04
Maternal granddam	0.31±0.05	0.19±0.05

same relatives. The differences are however not significant in view of their probable errors. These facts may be brought out very nicely by the use of graphical methods. Figure 3 shows the average milk yields of the daughters for fixed milk yields of the dam and granddam.

Figure 3 shows that as the milk yield of the dam increases the milk yield of the daughters on the average also increases. This increase is more pronounced than the increase found in the granddaughters' milk production with a corresponding increase in the granddam's milk production. On a numerical scale an increase of a thousand pounds in the dam's milk production results on the average in a corresponding increase of *367* pounds in the daughters' milk production. An increase of 1000 pounds

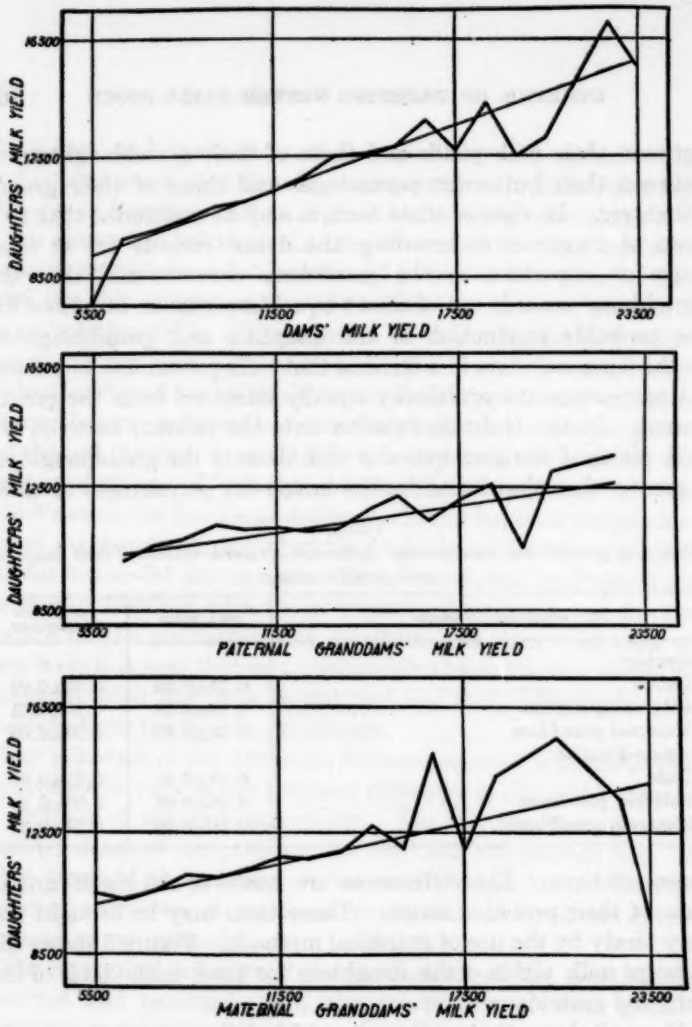


Fig. 3. Average Milk Yields of Daughters or Granddaughters for Dams or Granddams in the Guernsey Advanced Registry

The upper graph shows the relation between the daughter and dam. The middle graph the relation between granddaughter and paternal granddam. The lower graph the relation between granddaughter and maternal granddam. Examination of these graphs shows that for either type of ancestors an increase in the milk production results in an increased average production of their daughters or granddaughters. Contrasting the average production of the daughters for an increase in the production of the dams as compared with an average production of the daughters for a like increase in the production of the granddam, it is found that a given increase in the dam's production results in a much more marked average increase in the daughter's production, in fact nearly twice as great an increase in production as that found for granddam and granddaughter.

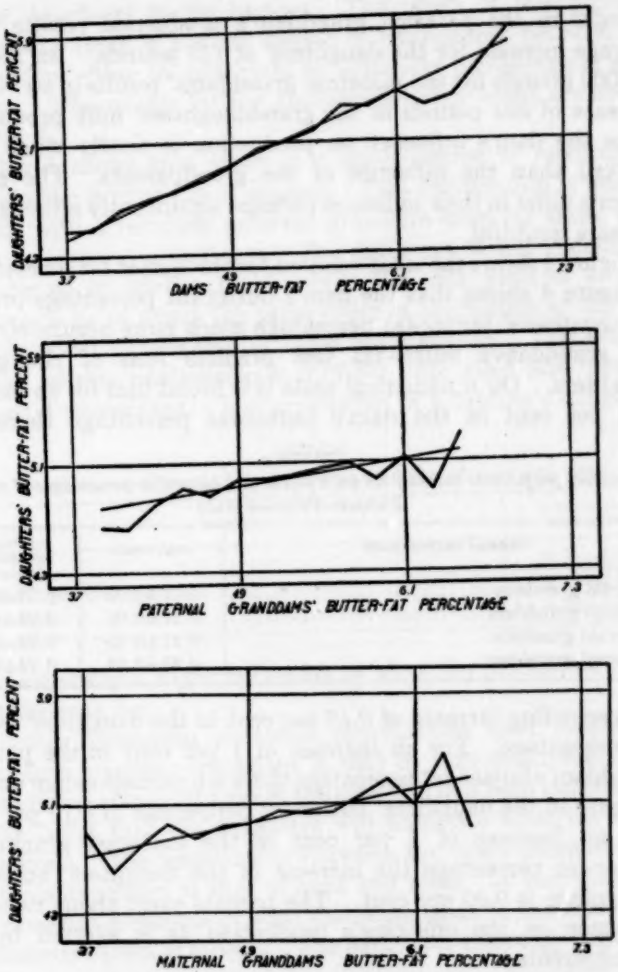


FIG. 4. RELATION BETWEEN THE BUTTER-FAT PERCENTAGES OF DAM OR GRANDDAM AND THEIR DAUGHTERS OR GRANDDAUGHTERS

These figures are drawn by the same scale. The parents production is shown in the abscissae of the figure, the ordinates represent the average production of the daughters and granddaughters. The figure shows clearly that there is a much greater relation between the dam's and daughter's butter-fat percentage as contrasted with that of granddam and granddaughter.

of milk in the paternal granddam's production results in an average increase for the daughters' of *143* pounds. An increase of 1000 pounds for the maternal granddams' results in an average increase of *224* pounds in the granddaughters' milk production. Thus the dam's influence on production is clearly much more marked than the influence of the grandparents. The grandparents differ in their influence perhaps significantly although this appears doubtful.

Figure 4 shows the same relation for the butter-fat percentages.

Figure 4 shows that the dam's butter-fat percentage predicts the daughters' butter-fat percentage much more accurately than the granddam's butter-fat test predicts that of the granddaughters. On a numerical scale it is found that for an increase of 1 per cent in the dam's butter-fat percentage there is a

TABLE 5

Correlation coefficients between the milk yields and butter-fat percentages of cousins, Holstein-Friesian breed

COMMON GRANDPARENT	MILK YIELD	BUTTER-FAT PERCENTAGE
Paternal grandsire	0.01±0.03	0.12±0.03
Paternal granddam	0.17±0.05	0.21±0.04
Maternal grandsire	0.21±0.02	0.22±0.02
Maternal granddam	0.23±0.04	0.24±0.04

corresponding increase of *0.43* per cent in the daughters' butter-fat percentage. For an increase of 1 per cent in the paternal granddam's butter-fat percentage there is a corresponding average increase in the daughters' butter-fat percentage of *0.16* per cent. For an increase of 1 per cent in the maternal granddam's butter-fat percentage the increase in the daughters' butter-fat percentage is *0.20* per cent. The parents exert about twice the influence on the offspring's production as is exerted by the grandparents.

Among the ancestral combinations used in dairy cattle breeding is that of cousins. The importance of this combination comes from the habit of giving a good deal of weight to production records of a brother's daughters in selecting a bull for a herd sire. The daughters of such a bull would, of course, be cousins

to the daughters of his brother. The study of this problem shows that there is a small degree of relationship between the production, either in milk yield or butter-fat percentage of such cousins. The results for the Holstein-Friesian breed are shown in table 5.

Table 5 shows that there is a small degree of relationship between the production of cousins. This relationship is less for cousins with a common paternal grandsire than for those with other common grandparents. The difference is not significant. The degree of relationship between the milk yield of cousins appears to be of about the same order of magnitude as that for the butter-fat percentages of these same cousins. The average amount of this relationship is *0.16* for milk yield and *0.20* for butter-fat percentage. The production of a brother's daughters

TABLE 6

Correlation coefficients between the productivity of aunt and niece

VARIABLE	CORRELATION COEFFICIENT
Milk yield	0.26±0.02
Butter-fat percentage	0.18±0.02
Butter-fat	0.29±0.02

is consequently a slight indication of what the production of the herd sire may be.

Another important ancestral combination used extensively in guiding breeding operations is that of aunt and niece. This combination comes into use in the selection of a herd bull because of the belief that his daughters will duplicate what his sisters have done. The relationship between the sire's daughters and his son's daughters is that of aunt and niece. A comparison of the production records of aunt and niece leads to the correlation coefficients shown in table 6. The data is for Guernsey Advanced Registry cattle.

Table 6 shows that there is a significant relation between the production of aunt and niece in milk yield, butter-fat percentage, or butter-fat. The differences between these correlations are of doubtful significance indicating that the relationship is about

of equal strength between the productions of aunt and niece in any of these items. Such results bear out the conclusion that milk yield, butter-fat percentage, and butter-fat are equally inherited and that the sire plays a fairly important part in this inheritance. The degree of relationship is practically that found for cousins. The record of an aunt or a cousin is consequently of about equal importance in indicating the probable production of any given cow.

The significance of these results for practical breeding may be stated thus: It is important in constructive breeding to take into consideration; first, the record of the bull's progeny; second, the record of his dam; third, but weighing it to a still less degree, the record of his sire's daughters or the bull's own half sisters. When these records are obtained and carefully considered they furnish about all the information which is valuable in selecting a herd sire. In view of the difficulty in getting many of these records it is an open question as to whether a herd sire should ever be purchased. It is in fact likely that more progress in building up the breed's production would be made by selecting the best producing and transmitting cow in the dairyman's barn to furnish the future herd sire. The significant records for a cow are in order of importance: first, her full sister's record; second, the record of her dam; third, the record of her half sister; fourth, the record of her granddam; fifth, the record of her aunt; and sixth, the record of her cousins. Other production records have doubtful significance in indicating what the cow's productive capacity will be. From these facts it is obviously much easier to select cows than it is to select bulls for prepotency in transmitting milk production or butter-fat percentage to their offspring.

Conformation has for many years been the major criterion by which dairy breeding stock is selected. This practice is based on the following ideas. First that conformation shows the probable production of the cow, second that conformation of the bull was transmitted to his offspring and therefore in a sense showed what this offspring's probable production would be. Within recent years data on this problem of some importance

have been collected and analyzed. The first problem does not in a sense belong in the genetic field. However, it is so intimately linked with breeding for production that it is important to review the evidence to form a foundation on which to study the breeding problem.

Conformation is found to be an important element influencing markedly the production of a cow. This influence is due to the fact that both conformation and milk production are closely associated with the age of the cow. If selection is practiced it is really a double selection based partly on conformation and partly on age. About half of the relation which exists between

TABLE 7

Conformation and productivity in Holstein-Friesian cattle under five and one-half years of age

CHARACTERS	MILK YIELD	
	Age variable	Age constant
Age	0.65	
Shoulder height	0.36	0.11
Hip height	0.36	0.14
Body length	0.58	0.26
Rump length	0.39	0.13
Body width	0.52	0.19
Thurl width	0.15	−0.06
Body girth	0.41	0.12
Weight	0.65	0.35

conformation and milk production is due to age and the other half is due to the differential growth which exists between cattle of the same age. Table 7 brings out this point for Holstein-Friesian cattle. The same general conclusion is also to be derived from a much more extensive and adequate series of data on Jersey animals.

The results of table 7 show that during the early years, age is of the most importance, much more important than conformation, in controlling milk yield. Body weight alone approaches the importance of age in controlling milk production. Besides this measurement other measurements indicating the relative size of the animal are also important. They are, however, closely

associated with weight so that in the long run weight appears to be the most important part of conformation to which consideration should be given in selecting dairy cows.

The score card data on Jersey cattle indicates the same general conclusion and supplements it in that it shows the udder condition in size and the characteristics of the milk veins to be very important elements of conformation in relation to milk production. The work of Aldrich and Dana bear out this conclusion in showing milk veins to be quite important elements of conformation as related to production. The data may, therefore, be summed up by saying that weight as a measure of size of the cow and the characteristics of the udder and milk veins are the important elements in indicating production.

The practice of the dairyman in selecting cattle for his herd on the basis of conformation in the belief that these animals would be capable of transmitting this favorable conformation to their offspring, has only recently been analyzed to establish whether this proposition were true or false. Thanks to the initiative of the American Jersey Cattle Club data are now available to show that the relative size of the cow is dependent to some degree on the relative size of the sire. If the sire is large the daughter is apt to be large. The degree of correlation which exists between the daughter's size and the sire's size is of about half the order of magnitude found between the milk production of a dam and that of her daughters. Thus to a limited degree the conformation of a sire is transmitted to his daughters.

The equally important hypothesis on which the dairyman manages his breeding herd, that the sire's conformation to some degree predicts the daughter's milk production, has now also been approached through the analysis of these same data collected by the American Jersey Cattle Club. The results of this analysis show that the item, weight of the sire, has a relation to the daughter's milk production. The larger the sire's weight the more the milk yield of the daughter. This relation is only about half of that found for the relation of the dam's and daughters' milk yield. It, furthermore, has no relation to the butter-fat percentage that the daughter is able to give. The other items of

conformation were not found to be particularly important in indicating the daughter's probable production. It is thus clear that conformation as a basis of selecting and breeding for milk production is of much less importance than the actual record of the ancestors within the first and second generations of the pedigree.

The significant points which genetics has thus far been able to analyze and present to the dairy cattle breeder interested in the intelligent betterment of his herd may be briefly reviewed as follows: The Mendelian analysis of coat color and of certain of the physical defects found in cattle has shown that by the use of of simple factor hypotheses it is possible to eliminate these defects and make for a more uniformly breeding animal whose characteristics will meet the breed association requirements. It has been possible to show that the production of the near relatives is much more important than that of those further removed. The significance of these results in selecting a bull for herd sire may be stated thus.

It is important to take into consideration: first, the record of the bull's progeny; second, the records of his dam; third, and weighting to a less degree the record of his sire's daughters or the bull's own half sisters. Unfortunately, it is very difficult to obtain these records on animals other than those raised by the breeder himself. In view of this difficulty it would frequently be to the breeder's interest to raise his own herd sire rather than to purchase an animal lacking such information. The more significant records for the cow are in order of importance: first, her full sisters' records; second, the record of her dam; third, the records of her half sisters; fourth, the records her granddams; fifth, the records of her aunts, and sixth, the records of her cousins. The other records have but doubtful significance in indicating what the cow's productive capacity will be. The variety of ancestors from which records may be obtained in selecting a cow make it clear that it is much easier to select females than it is to select males in replacing animals within the herd. Conformation is shown to have a relation to production, such that the size of the cow and the size of her udder and mammary development,

together with wedge shaped form are of importance in indicating the cow's probable productivity. It is further shown that the conformation of the sire is transmitted to his daughters to a limited degree. If the sire is large the daughter is apt to be large. The amount of relationship, however, on the whole is relatively small. The sire's conformation effects the daughters' conformation only in so far as the item of weight is concerned. It also appears that the most important item of the sire's conformation in affecting his daughters' milk production is that of weight. The larger the sire in weight, the more the probable milk production of the daughter will be. This relationship is, however, only about one-half of that found for the relation of the milk yields of dam and daughters so that it may be concluded that conformation is in no way a substitute for actual production records of the parents or grandparents of the animals purchased or bred in the maintenance of a productive herd.

REFERENCES

(1) Spillman, W. J. 1907 Report of committee on animal hybridizing. Amer. Breeder's Association Proceedings, iii, 184–189.

(2) Van Damme, C. 1908 Inheritance of coat color in the cattle of East Flanders. Rev. Gen. Agron., n. ser., iii, 363–365.

(3) Wilson, J. 1909 The colors of Highland cattle. Sci. Proc. Roy. Dublin Soc., n. ser., xii, pp. 66–76.

(4) Lloyd-Jones, O., and Evvard J. 1916 Inheritance of color and horns in blue-gray cattle. Iowa Sta. Research Bul. 30, 67a–106a.

(5) Gowen, John W. 1918 Studies in inheritance of certain characters of crosses between dairy and beef breeds of cattle. Jour. Agr. Research, xv, 1–57.

(6) Cole, L. J., and Jones, S. V. H. 1920 The occurrence of red calves in black breeds of cattle. Wis. Sta. Bul. 313, 1–36.

(7) Templeton, G. S. 1923 Unusual color inheritance. Jour. Heredity, xiv, 39–40.

(8) Detlefsen, J. A., and Yapp, W. W. 1920 The inheritance of congenital cataract in cattle. Amer. Nat., liv, 277–280.

(9) Cole, L. J. 1919 A defect of hair and teeth in cattle, probably hereditary. Jour. Heredity, x, 303–306.

(10) Yamane, J. 1915 On the inheritance of an aural abnormality in the Ayrshire cattle. Jour. Col. Agr. Tohoku Imp. Univ., vi, no. 7, 166–170.

Lush, J. L. 1922 An hereditary notch in the ears of Jersey cattle. Jour. Heredity, xiii, 8–13.

(11) Roberts, E. 1921 Polydactylism in cattle. Jour. Heredity, xii, 84–86.

PHYSICO-CHEMICAL FACTORS INFLUENCING CREAM RISING

II. RELATION OF PLASMA COLLOIDS TO PASTEURIZATION EFFECTS*

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It was shown in the first paper of this series (1) that variations in cream rising of cow's milk seem to be due primarily to certain changes in the dispersion medium, i.e., the plasma of the milk. In raw milk especially it was shown that the standardization of a given lot of skim milk with cream having either large or small fat globules resulted in cream layers of nearly uniform depth. Pasteurization, also, seemed to affect the milk plasma primarily, resulting in less exhaustive creaming and a closer packing of the fat globules in the cream layer.

The detrimental effect of pasteurization on creaming has been variously explained. According to Hunziker (2) there are two factors involved, (*a*) a coagulation of lactalbumin to form a sort of network that hinders the rise of fat globules, (*b*) a reduction in the ability of the fat to carry non-fatty constituents into the cream. So far as the first factor is concerned, the experiments of Freudenreich (3), Rupp (4), Weinlig (5), Grimmer, Kurtenacker, and Berg (6), Steiner (7), and Hiebenthal (8) indicate clearly that appreciable coagulation of lactalbumin does not occur under the influence of such time and temperature factors as seriously affect creaming. Rahn (9) has, in fact, effectively disposed of this theory by restoring the normal creaming capacity of boiled milk through the addition of colloidal material, such as gelatin or gum.

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So far as the second factor is concerned, the view of Weinlig (5) can be considered in harmony with that of Hunziker. Weinlig believes that the effects of pasteurization are to be explained by a diminution in the concentration of the skin or membrane of albuminous colloid around the fat globules, which is accompanied by a change in its composition and tenacity. It is difficult to secure experimental data to support or deny this view. Weinlig offers none in support of it. Palmer and Samuelsson (10) found that the gold number of the crude "membrane" material is greatly decreased (i.e. suspensoid stabilizing property increased) by boiling. Similar, as yet unpublished (11) results have been obtained in this laboratory by pasteurizing solutions of the "membrane" material. It is not possible to say definitely that this finding has a bearing on Hunziker's and Weinlig's theories, but it does not at first sight seem to support them. A substance whose protective qualities are increased by heat would not be expected to lose tenacity.

Rahn's recent studies (9, 12, 13) of the cause of pasteurization effects on cream rising has led him to revert to an earlier explanation (14). According to this view it is the grouping of the fat globules into clusters in raw milk which is the chief factor in creaming giving rise to normal cream layers. Pasteurization effects are explained by the breaking up of these clusters. Babcock and Russell (15) found that cluster disintegration began at 140°F. and was complete at 150°F. Rahn believes that single globules have relatively little tendency to rise in raw milk. He observed that raw milk shows many clusters of fat globules and a chaos of movement of the single globules while in heated milk the clusters are lacking and the movement of the single fat globules regular and their rate of rise more rapid than for the single globules in raw milk. Rahn's (9) observations on the rate of rise of single fat globules in milk has also led him to conclude that the speed is too slow to account for the cream layers that form on raw milk in twelve to twenty-four hours.

In none of the studies so far reported has an attempt been made to examine experimentally the relation of the various plasma constituents to cream rising or to ascertain the effect of heat on these constituents in relation to the cream rising phenomenon.

The success attained in securing normal cream layers in milks prepared by addition of suitable amounts of very rich cream to skim milk suggested a mode of attack on this problem not heretofore undertaken. The present paper reports some of the outstanding features of the experiments.

EXPERIMENTAL

Methods of procedure

The milk was fresh milk obtained from the University herd. Mixed milk from Holstein, Guernsey, Jersey, and Ayrshire cows was used unless the experiment called for high plasma solid (Jersey, Guernsey) or low plasma solid (Holstein) milk. The milk in most instances was standardized to contain 3.5 per cent fat as determined by the Babcock method. Pasteurization was effected by means of shotgun cans immersed in a vat of hot water.

Cream layers were determined in 200 cc. graduated cylinders. The height of the milk column in these cylinders was approximately 20 cm. Duplicate cylinders were placed at 0° and at 10°C. using a fireless cooker type of thermostat and melting ice for the low temperature and a vat of running tap water or an ice-cooled refrigerator for the higher temperature.

The percentage of fat in the skim milk layers was determined by analysis after drawing off the skim milk through a small hole near the base of the cylinders. From these data and the fat content of the whole milk the approximate concentration of fat in the cream was calculated by the formula*

$$C = \frac{100x - (100 - y)z}{y}$$

Where C = percentage of fat in the cream layer
x = percentage of fat in the milk
y = percentage of cream by volume
z = percentage of fat in skim layer

Specific gravity was determined by the lactometer or Westphal balance. Solids-not-fat were calculated using the tables of

*Author's note: The formula used for this calculation which was published in the first paper of this series, January, 1926, was in error. The formula as given above is correct.

Shaw and Eckles (16). Hydrogen ion concentrations were determined potentiometrically using the Bailey electrode, Leeds and Northrup Type K potentiometer, and normal calomel cell.

Casein was prepared from separated milk by the grain curd method (17) and purified by leaching for several days in frequent changes of distilled water adjusted to pH = 4.8. This casein was pressed dry, broken up into small pieces and used in the moist condition for the preparation of casein milks.

The casein milks were colloidal dispersions of calcium caseinate prepared by grinding the moist casein with suitable quantities of $CaCO_3$ and $Ca_3(PO_4)_2$ until a concentrated colloidal solution of calcium caseinate resulted. For example, in one experiment the casein from 155 pounds of skim milk was ground with 75 grams of $CaCO_3$ and 25 grams of $Ca_3(PO_4)_2$ and small amounts of water until a thick creamy paste resulted. This was diluted gradually with water with continued grinding until a moderately thin fluid resulted. Grinding was effected either by a colloid mill, or by a burr grinder. Excess $CaCO_3$ and $Ca_3(PO_4)_2$ were removed by a DeLaval No. 105 Clarifier. Casein concentrations were based on a nitrogen determination. In most cases a milk containing 9 to 10 per cent casein was used for the preparation of the various casein milks described below, suitable dilutions being made with water, lactose solution, cream, etc., to give products of the desired composition.

Whey was prepared by two methods, rennin and acid. Separator skim milk from mixed milk or from such breeds as it was desired to use to vary the concentration of whey proteins was treated with rennet extract or phosphoric acid. In the former clotting was allowed to take place at 32°C. and the curd cut and allowed to shrink at this temperature with very little agitation. The whey was drained off, clarified and analyzed for nitrogen by the Kjeldahl method. For the acid whey, casein was removed by the grain-curd method, the whey clarified and then neutralized to pH = 6.5 (determined potentiometrically) using a 5 per cent NaOH solution. After further clarification the whey was analyzed for nitrogen.

In certain experiments soluble salts of skim milk were removed

by dialysis. This was effected by placing the milk in collodion bags prepared in 200 cc. test tubes. These bags were prepared in the usual manner and then immersed in 1 per cent gelatin solution for fifteen minutes, followed by treatment with 2 per cent formaldehyde solution for the same length of time. The bags were then washed thoroughly with distilled water and kept immersed in water until used. The milk was allowed to dialyze in these bags under pressure, each bag being filled as full as possible and tied firmly with a rubber band. About 30 such bags were allowed to sink in cold distilled water placed in 5-gallon stone jars kept in the dairy refrigerator at 5° to 8°C. Dialysis was continued for twenty-four to thirty-six hours with several changes of water.

Experimental results

a. Effect of pasteurizing only the skim milk or cream. In order to ascertain the general relation of the plasma constituents to the effects of pasteurization on creaming, experiments were carried out first in which the plasma phase only was pasteurized and then used as the source of skim milk for the standardized product prepared from skim milk and cream. Several such experiments were conducted, the results of which are summarized in table 1. The data show that in most cases just as detrimental effects and in some cases much greater effects on cream rising are secured by pasteurizing the plasma phase of milk alone as when the whole milk is pasteurized. More striking results were secured in the first three experiments than in the later ones. The explanation is not clear. It may be stated, however, that the first three were a part of experiments carried out in 1922 and the others a year later. In spite of the variations in the extent of the destruction of cream rising ability, the data certainly point strongly to the conclusion that it is the plasma phase of milk which is affected chiefly in pasteurization and not the fat globules or substances associated with them through adsorption or other physico-chemical mechanism.

It is appreciated that these experiments call for others in which the cream only is pasteurized before mixing with the raw milk.

TABLE 1

Influence of pasteurizing the plasma on the creaming of raw milk

EXPERIMENT NUMBER	DESCRIPTION OF MILK	TEMPERATURE OF PASTEURIZATION	TEMPERATURE OF SKIM WHEN ADDING CREAM	VOLUME CREAM AFTER TWENTY-FOUR HOURS AT 0°C.
		°C.	°C.	per cent
1	Raw, whole*		32	13.5
	Raw skim + raw cream		23	13.3
	Pasteurized skim + raw cream	62	62	6.0
	Pasteurized skim + raw cream	67	67	2.0
2	Raw, whole		23	13.8
	Raw skim + raw cream		23	13.8
	Pasteurized skim + raw cream	62	23	1.0
	Pasteurized skim + raw cream	62	23	1.8
	Pasteurized skim + raw cream	67	23	0.5
	Pasteurized skim + raw cream	67	67	0.8
3	Raw, whole		23	13.5
	Raw skim + raw cream		23	12.9
	Pasteurized skim + raw cream	63	23	4.4
4	Raw skim + raw cream		23	14.0
	(Raw skim + raw cream) pasteurized	63	23	15.0
	Pasteurized skim + raw cream	63	23	9.0
5	Raw skim + raw cream		23	15.0
	(Raw skim + raw cream) pasteurized	63	23	9.0
	Pasteurized skim and raw cream	63	23	11.0
6	Raw Holstein whole		23	11.5
	Raw Holstein skim + raw cream		23	10.0
	Pasteurized Holstein	63	23	9.0
	(Holstein skim and cream) pasteurized	63	23	5.5
	Holstein skim (pasteurized) + raw cream	63	23	8.5
7	Raw Jersey skim + raw cream		23	13.8
	(Jersey skim + cream) pasteurized	63	23	9.2
	Jersey skim (pasteurized) + raw cream	63	23	11.0

* Experiments 1 to 5 were conducted with mixed milk.

Experiments of this nature were not carried out chiefly because of the difficulties involved in pasteurizing small lots of high fat cream so as to avoid rendering the fat, and at the same time produce effects due to heat alone. The problem was therefore approached indirectly, namely, by using cream from pasteurized milk.

TABLE 2

*Relative effect on cream rising of pasteurizing only the plasma or cream**

EXPERIMENT NUMBER	DESCRIPTION OF MILK	VOLUME CREAM AFTER TWENTY-FOUR HOURS AT 0°C.	FAT IN SKIM	APPROXIMATE FAT IN CREAM
		per cent	*per cent*	*per cent*
1	Raw, whole	16.0	0.50	19.3
	Raw skim + raw cream	13.5	0.55	22.4
	Pasteurized whole	10.5	1.55	20.2
	(Raw skim + raw cream) pasteurized	8.5	1.58	24.3
	Raw skim + cream from pasteurized whole	14.0	0.70	20.7
	Skim from pasteurized whole + raw cream	10.0	1.73	19.5
2	Raw, whole	14.3	0.55	21.5
	Raw skim + raw cream	14.3	0.55	21.5
	Pasteurized, whole	11.0	1.28	21.5
	(Raw skim + raw cream) pasteurized	9.3	1.70	21.1
	Raw skim + cream from pasteurized whole	14.0	0.50	21.8
	Skim from pasteurized whole + raw cream	5.3	2.20	26.9
	Pasteurized skim + raw cream	6.0	2.18	24.2

* We are greatly indebted to Prof. W. B. Combs, Division of Dairy Husbandry, University of Minnesota for assistance in carrying out these experiments.

Two experiments of this kind are shown in table 2. In both experiments pasteurization was at 63°C. for thirty minutes in shotgun cans. In the first experiment the raw cream used for standardization contained 62.5 per cent fat, and the cream from the pasteurized milk contained 56.75 per cent fat. In the second experiment the raw and pasteurized cream tested 63.25 and 58.25 per cent fat, respectively.

The results of the two experiments were uniform in showing that the use of pasteurized cream in standardizing a synthetic whole milk is not detrimental to the creaming if the skim milk has not been pasteurized. In other words the data demonstrate conclusively that it is the milk plasma and not the fat globules that are affected by heat so as to interfere with cream rising.

A careful study was made of the extent of fat globule clustering in connection with the second experiment of this group. Observations were made on the freshly prepared samples at the time they were set aside for creaming, and also on the cream layers and remixed samples after the twenty-four-hour creaming period. The method employed was to prepare a hanging drop and observe the fat globules as they flowed past the field of observation. Flowing was induced by capillary attraction between the edge of the concave depression in the slide and the cover slip carrying the hanging drop. No essential change in the extent of fat globule clustering of a single sample could be noticed after twenty-four hours standing in ice water. The cream and remixed milk were also essentially the same except that more large fat globules were observed in the cream than in the mixed milk.

The most important observation made was the complete lack of agreement between the creaming phenomena and clustering. For example, of the three samples showing good cream volumes and exhaustive creaming, namely, those in which the whole milk or skim milk was raw, there were no clusters whatever in the two samples prepared from skim milk and separated cream (either raw or pasteurized), while the raw untreated milk showed considerable clustering, it being estimated that one-third of the fat globules existed in pairs or cluster of three or more globules. A further lack of relationship between clustering and creaming was observed for the pasteurized samples, all of which showed some clustering, the maximum amount appearing in the sample which had the poorest cream layer.

These results are wholly irreconcilable with those theories which attempt to explain creaming variations by means of fat globule clustering or its absence.

b. Relative influence of plasma colloids and plasma crystalloids

on cream rising. Milk plasma is a mixture of various colloids and crystalloids. It is not to be expected that these two groups of substances exert a similar influence on cream rising of raw or pasteurized milk. However, the specific gravity of plasma is determined largely by the mineral salts and lactose in true solution so that it is a matter of speculation whether a synthetic milk consisting of a mixture of high test cream and plasma colloids could show normal cream rising properties. Moreover, the

TABLE 3

Cream rising of raw and pasteurized milk made from cream and dialyzed plasma

EXPERIMENT NUMBER	DESCRIPTION OF MILK	pH	SPECIFIC GRAVITY AT 15.5 °C.	CREAM VOLUME AFTER TWENTY-FOUR HOURS AT 0°C.	FAT IN WHOLE MILK	FAT IN SKIM LAYER	APPROXIMATE FAT IN CREAM LAYER
				per cent	*per cent*	*per cent*	*per cent*
1	(Natural skim + cream) raw.....	6.48		10.3	3.6	2.1	16.7
	(Natural skim + cream) pasteurized.	6.59		12.8	3.6	1.7	16.6
	(Dialyzed skim + cream) raw.....	6.91	1.010	15.0	3.6	2.1	10.8
	(Dialyzed skim + cream) pasteurized.	6.84	1.016	10.0	3.6	2.3	15.3
2	(Natural skim + cream) raw.....	6.49		15.0	3.9	1.0	20.3
	(Natural skim + cream) pasteurized.	6.32		9.0	3.9	2.2	21.1
	(Dialyzed skim + cream) raw.....	6.64	1.029	15.0	3.9	1.0	20.3
	(Dialyzed skim + cream) pasteurized	6.75	1.031	8.0	3.9	2.9	15.4
	Dialyzed skim (pasteurized) + raw cream.....	6.58	1.034	14.0	4.7	2.7	17.0

influence of mineral salts on both hydrophilic and hydrophobic colloids (especially the latter) is so great that very interesting possibilities regarding creaming phenomena are suggested by a determination of the effect of removing the crystalloids from milk plasma.

Two experiments of this nature are reported in table 3. The results are to be looked upon as approximations only so far as removing the mineral salts and lactose and other substances in

true solution are concerned. In the first place the dialyzed plasma (skim milk) can be said to have had its crystalloid content merely reduced very greatly. This is shown by the low specific gravity of the milk in experiment 1. Furthermore, the cream used for standardizing the fat content of the milk contained milk crystalloids although in relatively greatly reduced quantity. The cream used for experiments 1 and 2 tested 41 and 51 per cent fat, respectively.

The nearly normal specific gravity of the milk in experiment 2 was brought about by the addition of lactose to the dialyzed milk in quantity sufficient to restore the original lactose content. Approximately 5 grams of lactose were added to each 100 cc. of dialyzed plasma. The purpose was to determine the relative influence of the lactose and soluble mineral salts on the cream rising phenomena.

The conditions surrounding these two experiments were such that the rise of fat was very much delayed resulting in skim milk layers of relatively high fat content. It is to be noted, however, that the removal of either the mineral salts or the mineral salts and lactose in large measure by dialysis had no detrimental effect on the cream rising of the raw milk. The influence of pasteurization was also essentially the same for the unaltered and dialyzed milk.

An interesting fact brought out in this experiment is that cream volumes may be very good in spite of a high fat content in the skim layer. The approximate fat content in the cream layer is also surprisingly high in these cases, and emphasizes the part that packing plays in determining the volume of cream rising on a given volume of milk. The line of demarkation between the cream and skim milk layers, i.e., the true "cream line" was not very distinct in these experiments. This result was noted particularly in the case of the raw dialyzed plasma plus cream in experiment 1.

Although these two experiments can not be regarded as giving conclusive proof of the relative importance of the crystalloids and colloids of milk plasma in cream rising, the results certainly point very strongly to the conclusion that the raw milk creaming

phenomena and pasteurization effects are governed chiefly if not entirely by the plasma colloids and that the crystalloids play little if any part in cream rising problems.

TABLE 4

Physical and chemical properties of raw and pasteurized calcium caseinate milk

Experiment number	Source of fat	Treatment	Casein	Specific gravity at 15.5°C.	pH	Cream volume		Fat in skim		Approximate fat in cream	
						At 6°C.	At 12°C.	At 6°C.	At 12°C.	At 6°C.	At 12°C.
			per cent			per cent	per cent	per cent	per cent	per cent	per cent
1	Holstein	Raw	2.39	1.0075	6.77	3.0	4.5	2.97	2.47	20.6	26.0
		Pasteurized	2.39	1.0075	6.71	2.0	3.8	2.97	2.40	24.5	31.3
	Jersey-Guernsey	Raw	2.39	1.007	6.78	5.9	8.4	2.25	1.65	23.4	23.7
		Pasteurized	2.39	1.007	6.75	2.7	5.9	2.57	1.57	37.0	34.4
2	Holstein	Raw	2.93	1.0076	6.78	3.5	6.0	3.02	2.37	17.0	21.2
		Pasteurized	2.93	1.0076	6.73	2.6	5.1	2.90	2.25	26.0	26.8
	Jersey-Guernsey	Raw	2.93	1.0079	6.75	11.3	10.4	1.05	0.60	22.7	25.5
		Pasteurized	2.93	1.0079	6.72	3.5	5.3	2.40	1.52	33.8	38.9
3	Holstein	Raw	2.39	1.0239	6.12	2.5	6.8	2.80	2.63	29.8	15.4
		Pasteurized	2.39	1.0262	6.24	2.0	3.0	3.32	3.22	12.2	12.6
	Jersey-Guernsey	Raw	2.93	1.0262	6.02	9.0	5.5	2.48	2.58	14.9	21.1
		Pasteurized	2.93	1.0282	6.19	2.0	2.6	3.36	3.00	15.2	26.1
4	Holstein	Raw	2.39	1.0256	6.54	8.0	7.0	2.55	2.30	15.7	20.9
		Pasteurized	2.39	1.0280	6.54	4.0	6.3	3.00	2.27	18.0	23.4
	Jersey-Guernsey	Raw	2.93	1.0294	6.50	7.3	5.3	2.60	2.37	16.3	25.6
		Pasteurized	2.93	1.0314	6.50	5.8	7.5	2.65	1.90	19.0	24.6
5	Holstein	Raw	2.39	1.0278	6.59	6.0		2.35		17.4	
		Pasteurized	2.39	1.0298	6.59	2.0	2.0	2.90	2.60	23.0	32.5
	Jersey-Guernsey	Raw	2.93	1.0287	6.59	10.5	7.5	0.50		22.4	
		Pasteurized	2.93	1.0303	6.59	6.5	5.7	0.60		34.4	

c. Calcium caseinate as a factor in cream rising. The experiments reported in the preceding sections at once raise the question

as to which colloidal constituents of milk plasma are affected by heat so as to interfere with cream rising. As already pointed out Hunziker (2) holds that a coagulation of lactalbumin is probably responsible, in part, for abnormal cream rising of pasteurized milk. Casein, however, is by far the most abundant of the plasma colloids. The first experiments were therefore carried out for the purpose of determining its relation to the cream rising phenomena.

Five experiments in all were conducted using calcium caseinate milk. The data are summarized in table 4. Inasmuch as the experiments differed slightly in certain details, a brief description of the salient features of each seems advisable.

The preparation of the calcium caseinate dispersion was practically the same for each experiment. The method employed has already been described. In experiment 1 the casein concentration of 2.36 per cent is intended to represent Holstein milk. The concentration 2.93 per cent used in experiment 2 represents Jersey milk. In the remaining experiments milk was prepared representing both Holstein and Jersey. The concentrations selected are based on the data of Eckles and Shaw (18) on the relation of breed to composition of milk.

All the experiments were alike in that the cream used for standardizing the artificial whole milk was secured from both Holstein and Jersey-Guernsey whole milk. In experiments 1 and 2 small and large fat globule cream was added to the single plasma prepared for each experiment. In the remaining experiments Holstein cream was added only to Holstein plasma and Jersey-Guernsey cream to Jersey-Guernsey plasma. The fat content of the creams used for the synthetic whole milk in the various experiments was as follows:

EXPERIMENT	HOLSTEIN	JERSEY-GUERNSEY
1	51.5	56.5
2	51.5	56.6
3	58.0	63.0
4	54.0	60.0
5	52.0	60.0

It was intended that each lot of synthetic whole milk should have 3.5 per cent fat. The results did not deviate more than 0.1 per cent from this except in experiment 5 where the Jersey milk tested only 2.8 per cent fat and the Holstein 3.3 per cent.

In experiments 1 and 2 no attempt was made to control the specific gravity of the synthetic milk which accounts for the abnormally low values reported. In experiments 3, 4, and 5, however, the calcium caseinate was diluted with sufficient 10 per cent lactose solution as well as cream to give the milks a sugar content of 5 per cent for the Jersey milks and 4.65 per cent for the Holstein milks. This increased the specific gravity to values which approached more nearly that of normal milk.

The pH of the milk was not controlled in the first three experiments except in so far as the calcium caseinate was prepared as nearly neutral as possible. This accounts for the high pH of the milks in experiments 1 and 2 and the low pH of experiment 3. In experiments 4 and 5, however, the pH value of the milks was increased with a 10 per cent Na_2HPO_4 solution to approximately 6.5 to 6.6. This effectively overcame a slight tendency to coagulate which was noticed in the pasteurization of the milk in experiment 3.

The pasteurization procedure was the same for all experiments. When the milk in the shot gun cans had reached 63°C., the gentle agitation was discontinued throughout the holding time of thirty minutes except when temperature readings were taken at five-minute intervals.

The results were essentially the same in each experiment regardless of the pH, specific gravity, relative size of fat globules or variation in casein content as representing Holstein or Jersey milk. It was surprising that the specific gravity had so little effect. Since the variation of this property was brought about by lactose, it seems evident that the sugar content of milk has no influence on cream rising.

One outstanding feature of the results is the uniformly poor cream layers on the raw milk and the uniformly detrimental effect of pasteurization. The data show, of course, that one primary reason for the poor cream layers is the fact that in a

great majority of cases only a very small proportion of the fat rose on the casein milk. This can not be the sole explanation, however, inasmuch as very good cream layers were obtained in the dialysis experiments (table 3) in spite of a high concentration of fat in the skim milk layer.

It seems fairly obvious that calcium caseinate although the most prominent colloid in the plasma has relatively little to do with either promoting the rise of the fat globules or increasing the volume which they occupy as cream. It should be noted, however, that there is in general a duplication of the results secured in the previous study (1) in that pasteurization results in a less exhaustive creaming and a closer packing of the fat globules in the cream layer. It can not be decided definitely with the data now at hand whether this is an effect of pasteurization on calcium caseinate or to some other factor, but the indications point strongly to calcium caseinate as the constituent affected.

Another outstanding and in fact remarkable feature of these experiments is the increase in cream layers at 12°C. in comparison with 0°C. This result alone points conclusively to the fact that calcium caseinate is not the colloidal constituent of milk plasma which promotes normal cream rising. Natural milk always has a lesser cream layer at high temperatures than when kept cold.

A further fact not brought out in table 4 pointing to the same conclusion regarding the rôle of calcium caseinate in cream rising is that the line of demarkation between the cream and skim milk was very indistinct in many of these experiments especially in the cylinders containing pasteurized products. In many instances it was necessary to determine the cream layers by holding the cylinders before a strong light.

The experience with calcium caseinate suggests that if plasma colloids are responsible in the main for normal cream rising, it is the true hydrophils, lactalbumin and globulin that are concerned. The experiments reported in the next section throw light on this relationship.

d. Whey colloids as factors in cream rising. The experiments

to determine the influence of the whey colloids (chiefly lactalbumin and globulin) on creaming were carried out in essentially the same manner as the calcium caseinate experiments. The whey studies, however, naturally fall into two groups. In the first group, the results of which are presented in table 5, rennet whey of Holstein or Jersey-Guernsey or mixed origin was com-

TABLE 5

Whey colloids as a factor in cream rising on raw or pasteurized milk

EXPERIMENT NUMBER	SOURCE OF CREAM	PROTEIN CONTENT OF WHEY	SPECIFIC GRAVITY AT 15.5°C.	pH	TEMPERATURE OF PASTEURIZATION	CREAM VOLUME		FAT IN SKIM LAYER		APPROXIMATE FAT CONTENT OF CREAM	
						At 0°C.	At 12°C.	At 0°C.	At 12°C.	At 0°C.	At 12°C.
		per cent			°C.	per cent	per cent	per cent	per cent	per cent	per cent
1	Holstein	0.96	1.0247	6.34	Raw	11.5	5.0	2.00	1.90	15.1	33.9
		0.96	1.0247	6.42	63	16.3	7.8	1.95	1.75	11.5	24.3
	Jersey-Guernsey	0.96	1.0250	6.36	Raw	17.0	7.0	1.57	1.32	7.0	18.3
		0.96	1.0250		63	20.0	9.0	1.55	1.27	6.3	14.9
2	Jersey-Guernsey	1.08	1.0264	6.53	Raw	14.0	8.9	0.57	1.40	21.5	13.8
		1.08	1.0264	6.45	63	14.3	12.5	0.25	0.32	23.1	25.7
	Holstein	1.08	1.0263	6.48	Raw	13.6	7.5	0.80	1.60	21.4	26.9
		1.08	1.0263	6.46	63	15.8	13.8	0.40	0.45	20.1	22.6
3	Mixed		1.0264	6.45	Raw	13.0		0.80		21.6	
			1.0269	6.52	63	18.8		1.40		7.3	
			1.0279	6.53	67	17.0		1.60		6.9	
			1.0279	6.53	63*	10.0		1.85		18.4	

* Whey only pasteurized.

bined with Holstein or Jersey-Guernsey or mixed cream in various combinations to determine the effect of two levels of whey protein in connection with large and small fat globules. In particular, however, the pH of the synthetic milk in the experiments was nearly identical with that of fresh natural milk.

The second group shown in table 6 compares the use of acid and rennet whey as a plasma for synthetic milk in cream rising

studies. These milks differed from those in the first group by being somewhat acid in comparison with natural milk and therefore show in general the effect of increased acidity on cream rising.

In both groups of experiments pasteurization was carried out in shot gun cans. The "milk" was gently agitated until the

TABLE 6

Whey colloids as factors in cream rising of raw or pasteurized milk of relatively low pH

EXPERIMENT NUMBER	KIND OF WHEY	PROTEIN CONTENT OF WHEY	SPECIFIC GRAVITY AT 15.5°C.	pH	TEMPERATURE OF PASTEURIZATION	CREAM VOLUME AT 0°C.	FAT IN SKIM LAYER	APPROXIMATE FAT CONTENT OF CREAM LAYER
		per cent			°C.	*per cent*	*per cent*	*per cent*
1	Rennet	1.10	1.0259	6.12	Raw	7.6	0.82	36.0
		1.10	1.0259	6.12	63	7.6	1.45	28.3
		1.10	1.0258	6.12	Raw	23.5	0.02	10.0
		1.10	1.0258	6.13	63	26.6	1.01	10.4
2	Rennet		1.0245	6.26	Raw	8.0	1.70	24.2
			1.0245	6.32	63	12.0	1.50	18.2
			1.0245	6.29	63*	5.8	2.45	20.7
3	Rennet		1.0245	6.10	Raw	6.0	2.70	16.0
			1.0245	6.20	63	12.0	1.50	18.2
4	Acid		1.0241	6.06	Raw	10.0		
			1.0232	6.27	63	12.0		
			1.0233	6.28	67	10.0		
			1.0236	6.28	63*	10.0		

* Whey only pasteurized.

desired temperature of pasteurization was reached. There was also gentle agitation at five minute intervals during the 30-minute pasteurization period. In all cases the "milk" was cooled to 26°C. before placing in the creaming cylinders or mixing with cream in case the whey only was pasteurized.

Before pointing out the important features of the results of these experiments, it should be stated that pasteurization pro-

duced no visible effects on any of the whey milks. This was also true for the tests in which the whey alone was pasteurized before mixing with the cream.

In many respects the results with the whey milks were contrary to those obtained with natural or casein milks. It will be noted first that when the pH was normal the raw milk gave excellent cream layers and that these increased somewhat in proportion to the protein content of the whey. However, contrary to any previous result pasteurization gave a marked *increase* to the cream layer, even when the pasteurization temperature was sufficiently high to practically destroy cream rising on natural or casein milk. Similar but less marked increases in cream layers occurred when the more acid whey milks were pasteurized.

The effect of heating the whey milks on the rise of fat and its distribution between cream and skim milk layer is particularly interesting. The data show that with whey milks of normal pH (table 5) the result was a *more* exhaustive creaming and a *looser* packing of the fat in the cream. This is entirely opposite to the effect of heat on natural or casein milks which show a *less* exhaustive creaming and a *closer* packing of the fat in the cream. When the whey milks were somewhat acid, the results were not regular in these respects showing that other factors were operating which require further study for elucidation.

The object of comparing rennet and acid wheys was to determine whether the rennet enzymes remaining in the whey might be acting on the traces of casein added with the cream and thus be a factor in some of the surprising results secured. Unfortunately the acid and rennet wheys which are comparable are each somewhat acid in comparison with normal milk. There is nothing in the results, however, to indicate an effect exerted by rennet enzymes.

DISCUSSION

The results of this series of experiments give a particularly close insight into the fundamental factors operating in cream rising on raw and pasteurized milk. They show conclusively that plasma colloids are the chief, if not the only constituents of

milk that exert an influence in cream rising. It appears that the difference between the specific gravity of fat and of milk plasma as represented by lactose and salts has very little to do with the volume of the cream layer although the exhaustiveness of cream rising is much affected. However, it is also clear that exhaustiveness of creaming under uniform conditions is influenced so greatly by other factors that specific gravity differences are readily and often almost completely overcome.

Our study of the comparative effects of the two important colloids of milk, calcium caseinate and lactalbumin (as represented by whey) has led to surprising and unexpected results. The most prominent colloid of milk, calcium caseinate, is shown to be a very poor promoter of satisfactory cream rising or ample cream layers. When heated this colloid is even more detrimental to both the rise of cream and to a sufficient separation of the fat particles in the cream to give a deep cream layer. These results lead one to suspect that when an exhaustive cream rise occurs accompanied by a cream layer of satisfactory depth, it does so in spite of the presence of calcium caseinate; and when the detrimental effects of pasteurization are manifested, calcium caseinate is chiefly to blame for the result.

On the other hand the whey colloids are clearly promoters of satisfactory cream layers, and more surprising still their effectiveness is increased by pasteurization both with respect to more exhaustive creaming as well as cream layers of greater volume. Some of the older explanations of pasteurization effects on creaming thus become entirely untenable. The idea that a lactalbumin coagulation is responsible for the unsatisfactory cream layers on pasteurized milk is not substantiated. On the contrary whatever effect pasteurization exerts on lactalbumin is a gain rather than a hindrance to cream rising and cream layers.

At first sight it seems very surprising that calcium caseinate and lactalbumin are found to exert an opposing effect on cream rising phenomena. The exact cause of this requires further experimental study. Nevertheless the suggestion may be permitted that calcium caseinate and lactalbumin do not belong to the same class of colloids. As one of us (19) has recently

pointed out calcium caseinate has many of the properties of a suspensoid (hydrophobe) although also possessing important properties of an emulsoid (hydrophile). It is thus evident that the opposing effects of calcium caseinate and whey colloids on cream rising are in some way related to differences in the character of the colloids. It should be emphasized, however, that the questions involved in cream rising do not include that of emulsion stability. While there may be differences between calcium caseinate and whey proteins so far as emulsion stabilization is concerned both kinds of proteins are to be considered as excellent emulsifiers.[1] The creaming phenomena with which this study has dealt are cream rising and volume of cream layer. In general our results point to the conclusion that both exhaustiveness of rise of fat and greater volume of cream are promoted by the more truly hydrophillic colloids and depressed by colloids with hydrophobic properties. Pasteurization at 63°C. for thirty minutes increases the effectiveness of the former but decreases still further the effectiveness of the latter. When the emulsion contains both classes of colloids, it may be expected that the net result will be determined largely by the colloid present in greater concentration. This supposition in fact offers a plausible explanation of the general detrimental effect of pasteurization on creaming of whole milk.

It is not possible to reconcile the results of any phase of this study with those theories which seek to explain cream rising changes on the basis of fat globule clustering. Rahn (9, 12, 13) has recently emphasized these theories. Our studies show conclusively that fat clustering is not even uniformly coincident with either satisfactory cream volumes or exhaustive creaming and is not to be regarded as a primary cause of cream rising changes. The data presented in tables 1 and 2 in particular make it exceedingly difficult to believe that the mode of distribution of the fat globules could have played any significant part in the results. In fact as already pointed out in the presentation

[1] A comparison of the gold number of calcium caseinate and lactalbumin by Mr. Johnson (11) in this laboratory gives calcium caseinate a protective value (reciprocal of gold number) of 83 and of lactalbumin of 20.

of the data of the experiment shown in table 2, a careful examination of the wholly standardized milks of experiment 2, i.e., all those in which skim milk was mixed with either raw or pasteurized cream, failed to disclose clustering of the fat globules in any case; yet there was marked impairment of cream rising whenever the skim milk or the mixture of skim and cream had been pasteurized in spite of the fact that clustering was not entirely destroyed.

This experiment also fails to support Weinlig's (5) theory that pasteurization effects are to be explained by a change in the properties of the membrane surrounding the fat globules. While our data do not refute the assertion that some change occurs, it is obvious that it bears no relation to creaming.

CONCLUSIONS

1. The exhaustiveness of cream rising and the volume of the cream layer on cow's milk are determined largely by the plasma colloids.

2. Differences in density of fat and plasma do not determine the volume of cream and will not assure exhaustive rise of fat.

3. The calcium caseinate of cow's milk *hinders* cream rising and *does not promote* satisfactory cream volumes. These effects are increased by those pasteurization procedures which depress the cream rising of natural milk and give rise to smaller cream volumes.

4. The whey colloids of cow's milk *promote* both cream rising and satisfactory cream volumes. These effects are increased by the pasteurization procedures that give rise to unsatisfactory creaming of natural milk.

5. The detrimental effects of pasteurization on cream rising phenomena of natural milk are to be explained by the effect of heat on the predominating plasma colloid, calcium caseinate.

6. Changes in cream rising and cream layers on natural milk, especially those brought about by pasteurization, can not be explained solely by fat globule clustering or dispersion.

REFERENCES

(1) Palmer, L. S., and Anderson, E. O.: Journal Dairy Science, 1926, ix, 1.
(2) Hunziker, O. F.: Hoards Dairyman, lxi, 91 (1921).
(3) deFreudenreich, Ed.: Revue Generale du Lait, iv, 433, 1905.
(4) Rupp, P.: U. S. Dept. of Agr. Bull. 166, 1913.
(5) Weinlig, A. F.: Forsch. Geb. Milchwirtsch. u. Molkereiwes., 1922, ii, 127, 175.
(6) Grimmer, W., Kurtenacker, C., and Berg, R.: Biochem. Z., 1923, cxxxvii, 465.
(7) Steiner: Diss. Philos., Leipzig, 1910. Work cited by Rahn, O.: Milchw. Forsch., 1925, ii, 375.
(8) Hiebenthal: Cited by Rahn, O. Milchw. Forsch., 1925, ii, 376.
(9) Rahn, O.: Kolloid-Z., 1922, xxx, 110.
(10) Palmer, L. S., and Samuelsson, E.: Proc. Soc. Exp. Biol. Med., 1924, xxi, 537.
(11) Johnson, Otto: Thesis for M.S. degree, University of Minnesota, 1925.
(12) Rahn, O.: Deut. Milchw. Ztg., No. 27, 1925.
(13) Rahn, O.: Milchw. Forsch., 1925, ii, 383.
(14) Woll, F. W., Babcock, S. M., and Russell, H. L.: 20th Ann. Rep. Wis. Agr. Exp. Sta., 1903, p. 183.
(15) Babcock, S. M., and Russell, H. L.: 13th Ann. Rep. Wis. Agr. Exp. Sta., 1896, p. 81.
(16) Shaw, R. H., and Eckles, C. H.: U. S. Depart. of Agr. Bull. 134, 1911.
(17) Clark, W. M., Zoller, H. F., Dahlberg, A. O., and Weimer, A. C.: J. Ind. Eng., Chem., 1920, xii, 1163.
(18) Eckles, C. H., and Shaw, R. H.: U. S. Dept. of Agr. Bull. 156, 1913.
(19) Palmer, L. S., and Richardson, G. A.: 3rd Natl. Colloid Symposium Monograph, p. 112. Chemical Catalog Company, New York, 1925.

SOME OBSERVATIONS ON THE FREEZING POINT OF MILK*

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The cryoscopic method has been advanced by Hortvet (1) and others for the detection and determination of added water in milk. In the published work no mention has been made of the possible effect of dissolved substances in the water with which the milk was adulterated. Since the depression of the freezing point is dependent upon the number of dissolved particles in solution, it is important to know to what extent these dissolved substances would effect the cryoscopic index of milk.

Another factor that has not been sufficiently investigated is the effect of seasonal variation on the freezing point. Since the various constituents of milk vary to some degree with the season, it would be quite possible to expect a slight variation in freezing point due to seasonal conditions. Stoecklin (2) indicates that there is no seasonal variation.

This investigation was planned to include the two factors: (*a*) effect of dissolved substances, and (*b*) seasonal variation.

Hortvet (1) in an article on "The cryoscopy of milk" based on his report as Referee on Dairy Products at Convention of The Association of Official Agricultural Chemists, Washington, D. C., 1920 has made an exhaustive search of the literature on the subject. About twenty-five references are mentioned. These generally agree that the freezing point of normal herd milk falls within rather narrow limits, an average being about −0.55°C. One of these investigators states "as a result of tests applied on 2500 samples in four years that under all conditions milk does not vary in freezing point outside the limits of −0.545° and −0.565°" (3). Hortvet (4) examined a large number of samples of known purity from individual cows and herds. These were adulterated with various percentages of water and the amount

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determined by the cryoscopic method. His work shows that when the original freezing point is known, the amount of water added may be detected within 0.5 per cent. However, when −0.55° is taken as the freezing point, the added water was detected within less than 3 per cent.

Since the above mentioned report, several articles have been published (5, 6, 7) dealing largely with apparatus, precautions, effect of acidity and disease on detection of added water by this method. However, no mention is made of the effect of solutions with dissolved materials and the detection of the added water by the cryoscope.

In this work, the standard Hortvet cryoscope was used. The thermometer was standardized by the Bureau of Standards Sugar Solution Method recommended by Hortvet (8). Readings were made with a telescopic thermometer reader. The stirring was accomplished with a mechanical stirring device with an up and down motion delivering a stroke about once per second. All samples of milk were iced as soon as received.

I. INORGANIC SALTS AND SUCROSE AS A FACTOR

Since the depression of the freezing point of a liquid depends upon the number of particles in solution in the liquid, it would seem to follow that the accuracy of this method when applied for the purpose of detecting watering in milk would depend upon whether the water added was pure or a hard water containing considerable amounts of the mineral salts in solution. In other words, would the salts dissolved in a hard water overcome to a certain extent the effect of the added water? If this were the case, it would be possible to add an appreciable amount of such a water without causing too great a change in the freezing point.

An examination of the waters of Iowa (9) show many waters of a considerable degree of hardness. Some run as high as 9000 parts per million of dissolved minerals while values ranging from 3000 to 6000 parts per million are not at all uncommon. In order to determine the effect of such waters when added to milk on the freezing point of the milk, a water containing 1300 parts per million was added to the extent of 10 per cent. This water

when frozen against distilled water gave a freezing point which averaged consistently 0.02° lower. When distilled water was diluted with 10 per cent of this water, the freezing point was consistently 0.002°C. lower. Milk, when diluted with 10 per cent of this water, gave the same freezing point as milk diluted with 10 per cent distilled water. Table 1 shows these results.

From these data, we may conclude that waters with considerable dissolved minerals may be added to milk and that the salts present will not be effective towards changing the freezing point as determined by the cryoscope.

TABLE 1

ORIGINAL FREEZING POINTS OF FRESH MILK	FREEZING POINTS OF MILK 10 PER CENT VOLUME OF DISTILLED H_2O	FREEZING POINTS OF MILK DILUTED WITH 10 PER CENT VOLUME WATER
−0.543	−0.486	−0.48
−0.555	−0.495	−0.495
−0.551	−0.495	−0.495
−0.5507	−0.518	−0.5185
−0.554	−0.496	−0.496
	−0.523	−0.523*
−0.559	−0.4955	−0.496
−0.560	−0.500	−0.5005

* Four determinations were made on this sample without any variation of the freezing point.

The next step was the determination of the concentration of dissolved material necessary to become effective in varying the freezing point of milk and the relative effectiveness of different concentrations in overcoming the effect of added water. Several different solutions were used in the investigation of which magnesium chloride and sucrose solutions will be used to illustrate the results.

The various concentrations of solutions were based on calculations from Winter's formula,

$$W = \frac{(t - t')\ 100}{t}$$

where W is the percentage of added water, t is the freezing point of original milk and t' is the freezing point of the adulterated

sample. Not knowing what the freezing points of the original milks would be, −0.55°C. was taken arbitrarily for the value of t. With this as an arbitrary value for t, if $t - t'$ were 0.0275, then W equals 5, if $t - t'$ were 0.055, then W becomes 10, etc. When the various concentrations of salts in solution were frozen and the depressions inserted in the formula for $t - t'$, W was calculated and called the "water equivalent" because it was assumed when these solutions were added to milk that the

TABLE 2

Effect of added magnesium chloride solutions

(1) MOLAR CONCENTRATION	(2) EXPERIMENTAL FREEZING POINTS DEPRESSION OF SOLUTION	(3) WATER EQUIVALENT BY $W = \frac{(t - t')\,100}{t}$	(4) CALCULATED WATER EQUIVALENT OF 10 PER CENT VOLUME ADDED TO MILK	(5) MOLAR CONCENTRATION OF $MgCl_2 \cdot 6H_2O$ in MILK DILUTED 10 PER CENT	(6) APPARENT PER CENT WATER PRESENT $W = \frac{(t - t')\,100}{t}$
				0.0000 (Distilled H_2O)	10.1
0.00985	0.0417	7.2	0.72	0.000985	10.1
0.0295				0.00295	8.2
0.0920	0.240	43.6	4.36	0.00492	5.9
0.0690	0.330	60.0	6.00	0.0069	4.5
0.0887	0.419	76.2	7.62	0.00887	3.14
0.108	0.513	93.2	9.32	0.0108	0.88
0.128	0.601	109.3	10.93	0.0128	−0.17
0.148	0.686	124.7	12.47	0.0148	−2.1
0.167	0.792	144.0	14.40	0.0167	−3.5
0.187	0.872	158.5	15.85	0.0187	−5.2

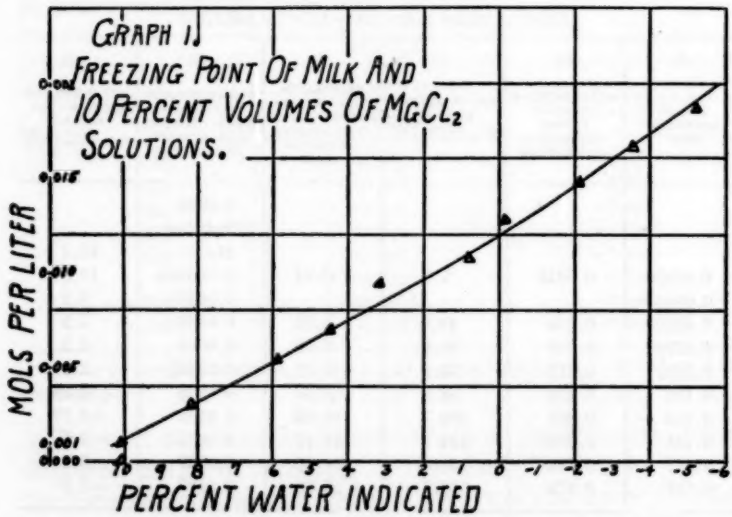
presence of the salt would cause a depression in the freezing point of the mixture which would compensate for the rise of freezing point caused by W amount of water added.

Assuming complete ionization for $MgCl_2 \cdot 6H_2O$, the following solutions were made up giving the various freezing point depressions found in column (2) of table 2. The different "water equivalents" as determined by

$$W = \frac{(t - t')\,100}{t}$$

where t is 0.55°, are found in column 3. The "water equivalent" values in column 6 were secured by diluting the milk with a 10 per cent volume of the magnesium chloride solutions. These dilutions were then frozen and the amount of water present was calculated by use of the Winter's formula. Figure 1 was plotted from these data.

In making figure 1 the data in column 5, table 2, was used for the ordinate while the values in column 6 were used for the abscissa. In calculating the values for column 6, the exact freez-



ing points of the original milk was substituted for t in Winter's formula. It should be noted that the smallest concentration of salt was not effective in lowering the higher freezing point of the adulterated milk since it froze at the same point as when 10 per cent distilled water was added. This concentration was equivalent to 1519 parts per million of dissolved magnesium chloride and should have counteracted the effect of 0.72 per cent of added water.

The next concentration of salt was effective inasmuch as it counteracted the effect of 1.9 per cent added water. This

concentration was equivalent to 3189 parts per million of dissolved material. A gradual lowering of freezing point continues until the salt is effective in overcoming completely the 10 per cent volume of water present from the solution. Additional concentrations lower the freezing point below that of the original milk and when calculated by the formula show a negative presence of water.

The largest concentration of salt should have been equivalent to 15.85 per cent added water according to column 4 and it

TABLE 3

Effect of added sucrose solutions

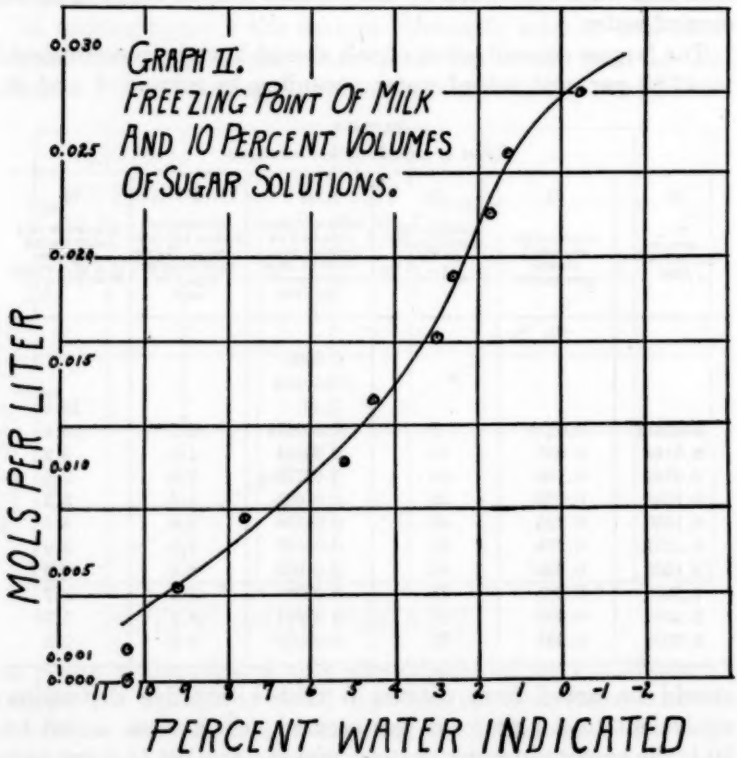
(1) MOLAR CONCENTRATION	(2) CALCULATED FREEZING POINTS DEPRESSION	(3) CALCULATED EQUIVALENT OF H_2O $W = \frac{(t - t')\,100}{t}$	(4) MOLAR CONCENTRATION OF SUCROSE SOLUTION IN MILK DILUTED 10 PER CENT	(5) CALCULATED WATER EQUIVALENT OF 10 PER CENT VOLUME ADDED TO MILK	(6) APPARENT PER CENT WATER PRESENT $W = \frac{(t - t')\,100}{t}$
	°C.				
			0.0000 (Distilled H_2O)		10.4
0.01479	0.0275	5	0.001479	0.5	10.4
0.0444	0.055	15	0.00444	1.5	9.2
0.0739	0.110	25	0.00739	2.5	7.6
0.1035	0.165	35	0.01035	3.5	5.2
0.1330	0.220	45	0.01330	4.5	4.5
0.1627	0.275	55	0.01627	5.5	3.0
0.1922	0.330	65	0.01922	6.5	2.6
0.2218	0.385	75	0.02218	7.5	1.7
0.2514	0.440	85	0.02514	8.5	1.25
0.2809	0.495	95	0.02809	9.5	−0.5

should be noted from column 6 that a negative depression equivalent to 5.2 per cent was secured, which when added to 10.1, the amount of water present, makes a total of 15.3 per cent water. This agrees quite well with the predictions.

It should be added here that the second concentration of milk and salt solution tasted decidedly salty.

Table 3 is similar to table 2 excepting that the freezing points depressions (column 2) of the various sucrose solutions were not obtained experimentally. These values were calculated on the

assumption that one molar weight of the sucrose in a liter solution would lower the freezing point 1.86°C. Using the calculated depressions in column 2, the equivalent water values found in column 3 were calculated by use of Winter's formula using 0.55 for *t*.



To make figure 2, the values in column 4, table 3, were used as the abscissa and those in column 6 as the ordinate. Again, it should be noted that the first concentration of sucrose was not effective in overcoming the effect of the water from the solution since it gave the same freezing point as when 10.4 per cent volume of distilled water was added. This concentration of sucrose is

equivalent to 5060 parts per million of dissolved material. The next larger concentration is equivalent to 15,180 parts per

TABLE 4

DATE 1924	ORIGINAL FREEZING POINT OF MILK	FREEZING POINT OF MILK AND 10 PER CENT BY VOLUME OF DISTILLED WATER	CALCULATED PER CENT H_2O BY $W = \frac{(t - t')\,100}{t}$	CALCULATED PER CENT H_2O BY $W = \frac{(0.55 - t')\,100}{0.55}$	pH
		Group A (Guernsey herd)			
4-26	−0.546	−0.489	10.4	10.3	
5-1	−0.544	−0.483	11.9	11.1	
5-6	−0.555	−0.495	11.2	10.9	
5-7	−0.553				6.604
5-8	−0.543	−0.486	10.5	10.3	6.689
5-20	−0.553	−0.491	11.2	11.2	6.532
5-21	−0.560				6.638
6-11	−0.5507				
6-12	−0.5448	−0.488	10.4	10.3	
6-13	−0.5527				6.531
6-15	−0.5695				6.276
		Group B (2 Jerseys)			
6-24	−0.5507	−0.495	10.11	10.1	6.5
6-25	−0.554	−0.496	10.47	10.5	6.503
6-27	−0.559	−0.496	11.3	11.4	6.57
6-28	−0.561	−0.500	10.87	11.1	6.65
6-30	−0.557	−0.495	11.13	11.3	6.56
7-5	−0.555				6.53
7-7	−0.5586	−0.503	10.02	10.1	6.59
7-9	−0.572	−0.512	10.5	10.9	6.55
7-10	−0.562	−0.498	11.3	11.5	6.61
7-12	−0.563	−0.506	10.1	10.4	
7-14	−0.557	−0.500	10.2	10.3	
7-15	−0.563	−0.504	10.5	10.7	
7-24	−0.578	−0.517	10.6	11.1	
8-19	−0.572	−0.514	10.6	10.5	
8-21	−0.578	−0.516	10.7	11.2	
8-26	−0.578	−0.518	10.4	10.9	

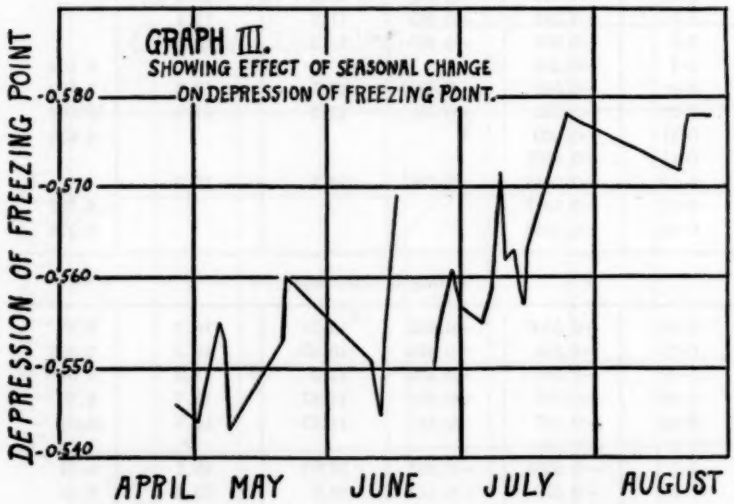
million and by calculation should have overcome the effect of 1.5 per cent added water. Experimentally, it succeeded in counteracting the effect of 1.2 per cent of added water. Addition of

larger concentrations caused the freezing point to lower until that of the original was reached, successfully counteracting the effect of the 10.4 volume of the water added.

Explanation of these phenomena may be found in the adsorption theory. However, this is a subject for further investigation, which is being carried on at the present time.

II. SEASONAL VARIATION AS A FACTOR

Work was done on milk from two known sources and covering a period of four months from April 26 to August 26. Source



A in table 4 was a Guernsey herd and was used from April 26 to June 15. Source B was a herd of two Jerseys and was continued until August 26. Samples were packed in ice as soon as received. Table 4 is a record of these results and figure 3 is made from some of the data in this table.

Figure 3 was made by plotting the freezing points of the original milk as the ordinate against the time of season as abscissa. It seems to indicate rather clearly that there is a tendency for the freezing point to lower as the season advances.

There is a considerable fluctuation in the freezing points of the samples from group A but group B is much more consistent and shows a distinct tendency to lower as the season advances. pH values were determined on a number of the samples from both herds. An examination of the data in table 4 will show that there is no correlation between them and the lowering of the freezing point as the season progresses. An explanation for the seasonal change may be due to a change in food. This explanation disagrees in part with some of the data collected by Hortvet (1). In this paper the work of one investigator is summed up in the following generalization:

> Milk, freshly drawn, from any variety cow, whether high or low breed, from whatever region of the country, from animals in stable or in pasture, whether poorly or substantially fed, whether drawn from a period near or remote from parturition, in winter or summer, morning or evening, whether the yield be scant or abundant, has a definite freezing point which varies but little around −0.550° although under various influences the chemical composition changes in enormous proportions.

Our data indicate that there is a consistent change in the freezing point during change of seasons.

III. CONCLUSIONS

The work on the factors affecting the freezing point of milk as determined by the cryoscopic method may be summed up as follows: (*a*) Equal volumes of distilled water and natural water of considerable hardness when added to milk give the same freezing point depression as detected by the cryoscopic method. (*b*) Magnesium chloride solutions containing at least 1519 parts per million may be added to milk without affecting the detection of the water in the solution by the cryoscopic method. Concentrations as great as 3189 parts per million counteract the effect of 1.9 per cent added. Greater concentrations counteract correspondingly greater quantities of water. (*c*) Concentrations of sucrose in solution as great as 5060 parts per million may be added to milk without affecting the detection of the water of the solution by the cryoscopic method; 15,180

parts per million counteracted the effect of 1.2 per cent added water. (*d*) There is a tendency for the freezing point of milk from the same herd to lower as the season advances from spring through the summer. (*e*) There is no correlation between the pH values and the freezing points of the milk during these seasons.

REFERENCES

(1) HORTVET, J.: Jour. Ind. and Eng. Chem., 1921, xiii, 198.
(2) STOECKLIN: Ann. fals., iv, 234.
(3) HORTVET, J.: Jour. Ind and Eng. Chem., 1921, xiii, 198.
(4) Ibid., p. 206.
(5) HORTVET, J.: Jour. Assoc. Official Agr. Chem., 1922, v, 470–84.
(6) BAILEY, E. M.: Jour. Assoc. Official Agr. Chem., 1923, vi, 429–34.
(7) GRONOVER: Z. Nahr. Genuss, 1924, xlv, 18–24.
(8) Standard Method of Milk Analysis, A. P. H. A., p. 33.
(9) Iowa Geological Survey, xxi, Annual Reports, 1910 and 1911.

A QUANTITATIVE FORM OF EXPRESSING PERSISTENCY OF MILK OR FAT SECRETION*

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It has been shown by work at this Station (1) that the secretion of milk and fat during the course of the lactation period rises for a time, reaches a maximum, and then gradually declines. It was pointed out that the rise in milk secretion becomes less and less as it approaches the maximum and the decline in this rise is exponential. After the maximum period of milk secretion, the curve of production declines and also follows an exponential law.

The exponential law, called by Lord Kelvin, the compound interest law which was found to express the change in the rate of milk secretion during the lactation period may be stated for our purpose as follows: When all other conditions are uniform the monthly milk or fat production during the lactation period after the maximum is passed, is a constant percentage of the preceeding month's production.

This law holds true to a remarkable degree with non-pregnant cows, but in the case of pregnant cows there is a more rapid decline during the last four months of pregnancy (2). This factor as well as seasonal changes of environmental temperature, nutrition, and management may cause slight changes in the rate of decline of milk and fat secreted (3).

The fact that the rate of increase and of decline of milk and fat secretion follows this important law of nature is of great value in the proper analysis of experimental results where milk and fat secretion is used as a criteria.

The purpose of this paper is to illustrate how this law may be employed in expressing, in a quantitative form, the persistency of milk or fat secretion of the dairy cow and its application to the analysis of experimental data.

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As indicated by the statement of the law, the percentage decline in milk flow from month to month should be fairly constant. However, some slight variations usually occur and therefore the best quantitative index of the persistency of production is the average per cent of persistency of secretion during the decline of milk flow. The method of calculating the average persistency of the group of non-pregnant cows will be used as an illustration of this method.

The milk production for the month of maximum production was 1052 pounds while the following month was 998 pounds. The percentage of the previous month's production may be obtained

TABLE 1

Persistency of milk secretion of non-pregnant cows

MONTH OF LACTATION	NUMBER ANIMALS	MONTHLY MILK PRODUCTION	PERCENTAGE OF PREVIOUS MONTH'S MILK PRODUCTION (PERSISTENCY INDEX)
2	920	1052	
3	912	998	94.86
4	923	938	93.98
5	914	879	93.71
6	917	833	94.76
7	912	792	95.07
8	904	752	94.94
9	905	715	95.07
10	906	676	94.54
11	852	650	96.15
12	653	617	94.92
Average			94.80

by dividing each month's production by the production of the preceding month. Thus,

$$\frac{998}{1052} \times 100 = 94.86 \text{ percentage of previous month's production}$$

The results are shown in table 1.

The average persistency index of this group of cows was 94.8 per cent. The persistency of fat secretion during the lactation period can be determined in a similar way. Due to the fact that the per cent of fat increases during the lactation as the milk

flow declines, the persistency of fat secretion is greater than is the persistency of milk secretion. In comparing individuals or groups of individuals it is important that comparisons be made only of either one or the other and not confuse persistency of milk secretion with persistency of fat secretion.

As working hypothesis it is assumed that each cow inherits a definite maximum persistency of secretion. Under favorable conditions she may closely approach her inheritance as regards this character but with poor feed and management, her potential persistency of secretion may not be fully expressed and the persistency index will be low.

One indication that there is a definite upper limit in the persistency of secretion is shown by the fact that overfeeding will not increase the persistency of secretion (4). The surplus nutrients consumed are stored on the body as indicated by an increase in live weight.

Having shown that the persistency of secretion is remarkably constant under controlled conditions, it may be used as a guide in evaluating the effect of unfavorable conditions on milk secretion. For example, in the case of the effect of pregnancy on the secretion of milk, it was noted that when cows are bred the third and fourth months of lactation, the last three months of lactation show a greater decline than the previous month's decline. This indicates the extent of the effect of pregnancy on milk secretion. At the same time it shows that these last months can not fairly be included in determining the average persistency index.

The frequency of milking is another factor which would affect the average persistency index of the dairy cow. Quantitative data on the effect of four, three, and two times a day milking on the persistency index will be of great value. However, unless the same cows are used in each classification, there will be danger of getting a more persistent group of animals in the classes with the greatest frequency of milking.

From the preceding discussion it will be seen that there are two factors in operation. To obtain a persistency index which will best represent the inheritance of the animal for persistency of milk secretion, it is necessary to exclude that part of the lactation

which is markedly influenced by environmental factors which may be noted by marked deviation from the average. On the other hand, the amount of deviation from the average is an excellent index of the effect of environmental factors of various kinds on milk secretion.

RELATION BETWEEN MAXIMUM MONTH'S PRODUCTION AND TOTAL PRODUCTION

The two factors chiefly concerned in total milk and fat production are the yield during the maximum month (a month is used as a convenient unit) and the persistency of production or the rate of decline. At a given maximum month's production, the cause of variations in total yield of milk or fat are due to the variations in the rate of decline. This being true, it is possible to obtain an index of persistency by determining the ratio between the total yearly milk or fat production and the maximum month's production. For example, if the total yearly production were 600 pounds of fat and the fat production during the maximum month were 50 pounds, then the ratio $\frac{600}{50}$ would be 12. This would mean that each month's production was, on the average, 50 pounds of fat and that the cow was 100 per cent persistent. In other words, a ratio of 12 for a yearly record would be equivalent to 100 per cent of persistency. For a ten months or three-hundred-and-five-day record, a ratio of 10 would be equivalent to 100 per cent persistency.

In order to interpret, in terms of persistency, ratios obtained which are less than 12 or 10 as the case may be, table 2 was prepared. It shows the rate of decline of fat production with varying percentages of persistency. Taking 100 pounds of fat as the maximum for the second month, the fat secreted each month with varying rates of decline in persistency from 100 per cent down to 85 per cent are presented. A slight possible error, may be introduced by assuming that the first and third months would be similar. It was used because there are indications that a close relation exists between the rate of increase of milk secretion and the rate of decline of milk secretion during the lactation period.

The total yield of fat for the ten months and also for the year

TABLE 2

The decline of fat secretion with varying per cent of persistency

MONTH OF LACTATION	PER CENT OF PERSISTENCY															
	100	99	98	97	96	95	94	93	92	91	90	89	88	87	86	85
	lbs. fat	*lbs. fat*	*lbs. fat*	*lbs. fat*	*lbs. fat*	*lbs. fat*	*lbs. fat*	*lbs. fat*	*lbs. fat*	*lbs. fat*	*lbs. fat*	*lbs. fat*	*lbs. fat*	*lbs. fat*	*lbs. fat*	*lbs. fat*
1	100	99.00	98.00	97.00	96.00	95.00	94.00	93.00	92.00	91.00	90.00	89.00	88.00	87.00	86.00	85.00
2	100	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
3	100	99.00	98.00	97.00	96.00	95.00	94.00	93.00	92.00	91.00	90.00	89.00	88.00	87.00	86.00	85.00
4	100	98.01	96.04	94.09	92.16	90.25	88.36	86.49	84.64	82.81	81.00	79.21	77.44	75.69	73.96	72.25
5	100	97.03	94.12	91.27	88.47	85.74	83.06	80.44	77.87	75.36	72.90	70.74	68.15	65.85	63.60	61.41
6	100	96.06	92.24	88.53	84.94	81.45	78.07	74.80	71.64	68.58	65.61	62.74	59.97	57.29	54.70	52.50
7	100	95.10	90.39	85.87	81.54	77.38	73.39	69.48	65.91	62.40	59.05	55.84	52.77	49.84	47.04	44.37
8	100	94.15	88.59	83.30	78.28	73.51	68.99	64.61	60.63	56.79	53.15	49.70	46.44	43.36	40.45	37.71
9	100	93.21	86.81	80.80	75.14	69.84	64.85	60.09	55.78	51.68	47.83	44.23	40.87	37.73	34.79	32.05
10	100	91.35	85.08	78.34	72.14	66.34	60.96	55.89	51.32	47.03	43.05	39.37	35.96	32.82	29.92	27.24
Total	1,000	963.84	929.27	896.20	864.67	834.51	805.86	777.80	751.79	726.65	702.59	679.83	657.60	636.58	616.48	587.26
Ratio of $\frac{\text{total}}{\text{maximum}}$	10.00	9.63	9.29	8.96	8.64	8.34	8.05	7.77	7.51	7.26	7.02	6.79	6.57	6.36	6.16	5.87
11	100	90.44	83.38	76.02	69.25	63.03	57.30	51.97	47.21	42.79	38.74	35.04	31.65	28.56	25.73	23.16
12	100	89.54	81.71	73.74	66.48	59.87	53.86	48.34	43.44	39.04	34.87	31.18	27.85	24.84	22.13	19.68
Total yearly fat production	1,200	1,143.82	1,094.36	1,045.96	1,000.40	957.41	916.84	878.11	842.44	809.38	776.20	746.05	717.10	689.98	664.34	640.11
Ratio of $\frac{\text{total}}{\text{maximum}}$	12.00	11.43	10.94	10.45	10.00	9.57	9.16	8.78	8.42	8.09	7.76	7.46	7.17	6.89	6.64	6.40

are given with the ratio of the total fat production for the period to the maximum month's production. These results are plotted in figure 1. From the figure it is possible to determine the per-

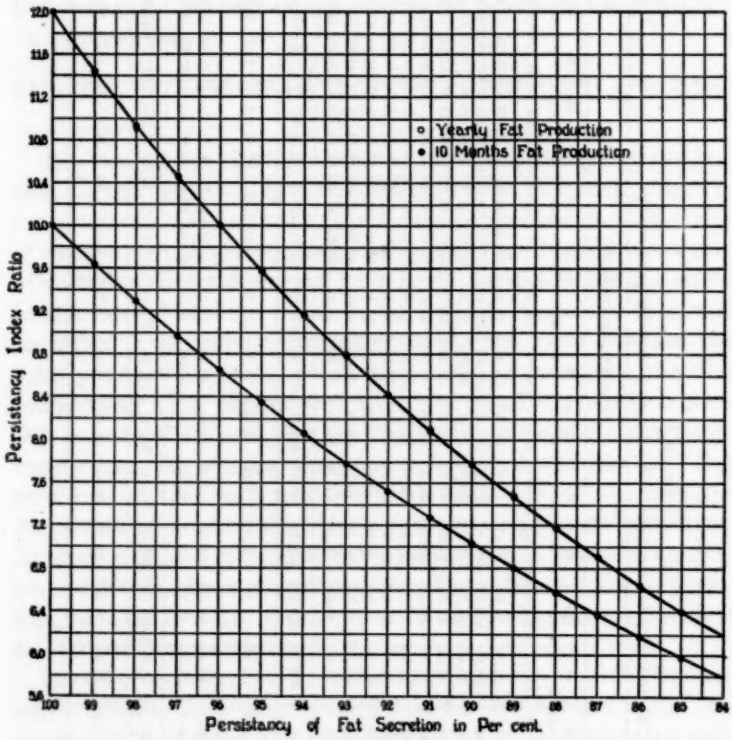

FIG. 1. RELATION BETWEEN PERSISTENCY OF FAT SECRETION IN PER CENT AND THE PERSISTENCY INDEX RATIO

It was plotted from the data in table 2. The persistency index ratio is the relation between the maximum month's fat production and the total fat production. The persistency of fat secretion in per cent is obtained by dividing each month's production by the production of the preceding month.

centage of persistency from any given value for the ratio either of the ten months or years production. For example, a ratio of 9.6 for a yearly record is equivalent to 95.1 per cent of persistency, a ratio of 10.8 is equivalent to 97.8 per cent. For a ten-

months production, a ratio of 9.2 is equivalent to 97.7 per cent of persistency.

THE PREDICTION OF TOTAL PRODUCTION

The relation of the maximum month's production and persistency of secretion to total yearly production may be used in predicting total yearly production from the first part of the record. The accuracy of the method depends on the uniformity of the rate of decline of milk secretion. If the environmental conditions are favorable and the decline in production is fairly uniform, an

TABLE 3

	DAYS	POUNDS FAT	PERCENTAGE OF PREVIOUS MONTH'S PRODUCTION
January	17	54.75	
February	28	106.40	
March	31	102.28	99.5
April	30	100.68	98.4
May	31	102.46	101.7
June	30	92.74	90.5
			(Ave. 4 months, 97.5)
July	31	94.35	101.7
August	31	85.36	90.4
			(Ave. 6 months, 97.0)
September	30	75.02	87.8
October	31	72.32	96.3
November	30	70.98	98.1
December	31	73.34	103.3
January	14	33.45	
		1,064.12	96.77 (ave.)

accurate prediction can be made. The method can be illustrated by the record of a 1000-pound cow selected at random (table 3).

Let us assume first that only the first four months (after the maximum) are known. To estimate the total production, it is first necessary to determine the average persistency index during this period (obtained by determining the percentage of the previous production month by month). In this case it is 97.5. The persistency index is converted to the corresponding ratio by reference to figure 1. The ratio of 97.5 is 10.6. The ratio is

then multiplied by the production during the maximum month resulting in the estimated total yearly production. Thus,

$$106.4 \times 10.6 = 1127.8 \text{ pounds of fat}$$

Referring to the actual production, 1064 pounds of fat, the estimated production is found to be 6 per cent too high. If the first six months are used, the estimated production would be 1106.6 pounds of fat or 4 per cent too high.

VARIATION IN PERSISTENCY OF DAIRY COWS

To obtain an idea of the variation in the persistency of dairy cows, table 4 is presented. The relation between the maximum month and total yearly production is shown. It will be noted that there is considerable range in yearly production with cows producing the same amount of fat during their maximum month. As an illustration, the total production of the cows averaging 52.5 pounds of fat during their maximum month may be cited. It will be seen that the extremes of production are 363 pounds and 663 pounds, a difference of 300 pounds of fat. It is hardly conceivable that persistency would cause such a large difference. The numbers of these cows is limited. However, 113 cows or 12.2 per cent of the 928 cows in the group produced less than 425 pounds of fat, 209 cows or 22.5 per cent produced less than 450 pounds, 334 cows or 35.9 per cent produced less than 475 pounds of fat and 470 cows or 50.6 per cent produced less than 500 pounds of fat. On the other hand, 56 cows or 6 per cent produced above 575 pounds of fat, 151 or 16.3 per cent produced above 550 pounds of fat, and 283 cows or 30.5 per cent produced above 525 pounds of fat. There is a variation of more tha 100 pounds of fat by 56 per cent of the animals having an average month's maximum fat production of 52.5 pounds. The importance of persistency of production as related to total yearly production is strikingly shown. It also illustrates the importance of having a quantitative method of expressing this important characteristic.

TABLE 4

The relation between the maximum month's and total production, Guernsey cattle

FAT PRODUCTION (MAXIMUM MONTH)	TOTAL YEARLY FAT PRODUCTION, POUNDS																									
	362.5	387.5	412.5	437.5	462.5	487.5	512.5	537.5	562.5	587.5	612.5	637.5	662.5	687.5	712.5	737.5	762.5	787.5	812.5	837.5	862.5	887.5	912.5	937.5	962.5	982.5	Total
pounds																											
32.5	13	1																									14
37.5	43	48	32	7	2																						132
42.5	46	99	108	102	64	17	1																				437
47.5	26	59	109	122	154	121	86	30	3	1																	711
52.5	10	40	63	96	125	136	175	132	95	39	13	2	2														928
57.5	6	14	37	63	63	91	107	145	126	113	81	43	9	1													899
62.5			12	24	24	48	79	79	82	93	100	97	84	45	14	2											783
67.5			0	11	10	16	25	34	45	47	70	77	64	92	67	31	11	3									603
72.5			2	2	5	8	12	13	17	28	38	40	32	46	49	45	30	11	6	3							387
77.5						1	7	7	10	9	10	17	22	14	17	19	29	25	21	20	5	4					237
82.5								0	6	1	6	4	20	3	7	14	8	16	13	10	14	7	1	1			131
87.5								1	1	1	3	6	3	1	3	7	7	8	7	6	3	4	3	7	1	1	73
92.5													3	1	1	3	4	0	5	3	7	2	6	4	2	2	43
97.5															2	1	1	0	1	3	0	0	0	0	0	1	9
102.5																	1		0	1	1	0	0	1	1		5
107.5																			4								4
Total.....	144	261	363	427	447	438	492	441	385	332	321	286	239	203	160	122	91	63	57	46	30	17	10	13	4	4	5,396

Correlation coefficient 0.761 ± 0.004.

USES FOR PERSISTENCY INDEX

In comparative feeding trials it is important to determine the persistency of production (as indicated by previous lactations) of each cow and so group the lots of cows as to include animals of similar persistency in each lot. It seems possible that by increasing the sizes of the lots of cows and selecting the lots of cows carefully on the basis of their persistency of secretion, that continuous feeding trials without reversal would be practical. The effect of long continued experiments in which the effect of the previous feed would be eliminated as a factor are highly desirable. This would bring feeding trials of dairy cattle to a more exact basis and would be similar to the present method of feeding experiments with small animals with growth and reproduction as an index of the nutritive completeness of the feeds in question.

Are certain rations more efficient in maintaining the persistency of production of milk and fat, and permitting normal reproduction? The answer to this question can only be obtained by long continued, non-reversed feeding trials with cows evenly grouped according to their persistency of production in addition to the usual factors considered. The influence of various systems of management on persistency of production may also be studied.

From the standpoint of improvement of the milk and fat producing capacity of dairy cattle, the persistency index gives a quantitative value for persistency which can be used as a guide in the selection of breeding stock. Heretofore, the qualitative expression has been that a certain cow was persistent or that another lacked persistency, but this method enables one to say in a quantitative form that a cow is 97 per cent persistent or 93 per cent persistent as the case may be. The tremendous effect of persistency on the total yield of milk and fat which was shown in this paper, clearly indicates the need of considering persistency in the selection of breeding stock.

SHORT-TIME TEST LACKS PERSISTENCY INDEX

There has been much discussion of the merits and demerits of the short time test during the past few years. It appears to the

writer that the greatest criticism of such a short test is that it does not furnish an index of the persistency of the cow. If these two characteristics are not inherited together (high maximum production and persistency of production) and there is much evidence that such is not the case,[1] then continued selection for high maximum production is sooner or later going to produce cows excelling in the production of milk for a short time but lacking in the ability to continue that production for a full lactation period. The effect of such a course would be to materially decrease long time production.

RELATION OF CONFORMATION TO PRODUCTION

The question of conformation or type and production is one that has been much discussed but is a problem that has received little serious attention. Recently there has been an attempt to determine the relation between various external measurements and production. It seems reasonable that there should be a fairly close relation between a combination of these measurements and maximum production (seven to thirty days) because size of body and udder are needed for large production. Persistency of production, which so vitally influences long-time production, has few if any external signs by which its presence or absence may be indicated. For this reason it would appear that conformation can not bear a closer relation to yearly production than to maximum production.

SUMMARY

1. When all other conditions are uniform, the monthly milk or fat production during the lactation period after the maximum is passed, is a constant percentage of the preceding month's production.

2. Pregnancy, seasonal changes of environmental temperature, nutrition, and management may cause slight changes in the rate of decline of milk and fat secreted.

[1] An examination of table 4 shows that persistency of production and maximum of production are not closely correlated. Both low and high maximum production are found with low persistency and the same is true of high persistency.

3. Two methods for the quantitative determination of persistency of milk secretion are explained with a discussion of their relative merits.

4. The use of a combination of these methods in predicting total yearly production from the first part of the record by the use of the maximum month's yield and the persistency of production shown during that period was illustrated.

5. The uses of a quantitative index for persistency of secretion of milk or fat by dairy cattle in relation to problems of breeding, feeding, and management were indicated.

REFERENCES

(1) Turner, Chas. W., Ragsdale, A. C., and Brody, S. 1923 How the advance of the period of lactation affects the milk flow. Jour. Dairy Sci., vi, no. 6, 527.

Brody, S., Turner, Chas. W., and Ragsdale, A. C. 1924 The relation between the initial rise and subsequent decline of milk secretion following parturition. Jour. Gen. Physiol., vi, no. 5, 541.

(2) Ragsdale, A. C., Turner, Chas. W., and Brody, S. 1924 The effect of gestation upon lactation in the dairy cow. Jour. Dairy Sci., vii, no. 1, 24.

Brody, S., Ragsdale, A. C., and Turner, Chas. W. 1923 The effect of gestation on the rate of decline of milk secretion with the advance of the period of lactation. Jour. Gen. Physiol., v, no. 6, 777.

(3) Turner, Chas. W. 1923 Seasonal variations in milk and fat production. Jour. Dairy Sci., vi, no. 3, 198.

(4) Turner, Chas. W. 1924 A study of the relation between feed consumption and milk secretion. Jour. Dairy Sci., vii, no. 6, 535.

INCREASED PRODUCING ABILITY IN DAIRY COWS DUE TO TEST CONDITIONS*

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Men who have handled large herds of cattle where official testing is carried on are generally close observers, and the statement has been made that when cows or heifers are placed on test they usually show development and increased producing ability as a result of the feeding and handling they receive during the period of the test. It would appear to be difficult to calculate the amount of development which might be attributed to the changed conditions surrounding the cow while on test, but a recent study of Guernsey and Jersey records throws some light on this matter.

It was originally planned to study the effect of age on production of butterfat by using only records of the cows having two or more tests. The thought was that the first record measured the cow's ability at a certain age, and the second record showed her ability at a later age. The difference would appear to be due to the increase in age between the tests. That this was not true was revealed when no indication was found showing when increasing age failed to improve production. It did not seem logical that age alone could be the cause of this continued improvement in the producing ability of cows after they had reached maturity, and for this reason the data were studied in a different manner.

Assuming that there was another factor besides age to be considered, the re-entry records were laid aside, and the initial records alone were sorted according to the ages at which the records were made. This gave the average production for initial-record cows at various ages, and since none of these were re-entry records the resulting averages for various age groups

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indicated the change in production brought about by age alone. Using these figures as a basis of the relationship of records of different ages, it was possible to adjust records to a comparable basis.

TABLE 1

Comparison of average initial and retest records of Jersey cows with initial records made while two to two and one-half years old

NUMBER OF COWS	AVERAGE INITIAL BUTTERFAT RECORD MADE AT 2 TO 2½ YEARS OLD	AVERAGE RE-ENTRY RECORD		INCREASE DUE TO AGE	PORTION OF RE-ENTRY RECORD DUE TO DEVELOPMENT	
		Age when made	Butterfat			
	pounds	*years*	*pounds*	*pounds*	*pounds*	*per cent**
240	384	3 – 3½	475	37	55	11.5
218	395	3½– 4	494	59	40	8.1
168	367	4 – 4½	496	72	57	11.5
152	391	4½– 5	553	92	70	12.7
105	388	5 – 5½	535	94	53	9.9
91	375	5½– 6	570	103	92	16.1
64	381	6 – 6½	548	108	59	10.8
43	382	6½– 7	561	108	71	12.7
36	365	7 – 7½	563	103	95	16.9
35	366	7½– 8	567	104	97	17.1
13	364	8 – 8½	589	103	122	20.7
12	332	8½– 9	549	94	123	22.4
3	322	9 – 9½	441	91	28	6.4
6	363	9½–10	632	102	167	26.4
2	279	10 –10½	490	79	132	26.9
2	393	11 –11½	732	95	244	33.3
2	365	13 –13½	528	88	75	14.2
1	396	14½–15	567	57	114	20.1
1	396	15½–16	683	57	230	33.7

* Weighted average per cent of this group is 11.9.

The study of the cows with two or more records was then resumed, and all records grouped according to ages. Then the animals with yearling records were regrouped according to the ages at which they were retested. In a like manner those with initial records made as junior two-year-olds, senior

two-year-olds, junior three-year-olds, etc., were sorted. The average initial record of each subgroup of cows was compared with the average initial record of all cows of the same age as previously determined. The next step was to calculate a record for these same cows at the age at which they were retested. This was done by adjusting the average initial record for that

TABLE 2

Comparison of average initial and retest records of Guernsey cows with initial records made while two to two and one-half years old

NUMBER OF COWS	AVERAGE INITIAL BUTTERFAT RECORD MADE AT 2 TO 2½ YEARS OLD	AVERAGE RE-ENTRY RECORD		INCREASE DUE TO AGE	PORTION OF RE-ENTRY RECORD DUE TO DEVELOPMENT	
		Age when made	Butterfat			
	pounds	*years*	*pounds*	*pounds*	*pounds*	*per cent**
46	424	3 – 3½	501	31	46	9.2
106	449	3½– 4	546	54	43	7.9
50	427	4 – 4½	541	70	44	8.1
70	435	4½– 5	552	74	43	7.8
36	418	5 – 5½	596	91	87	14.6
34	416	5½– 6	574	91	67	11.7
34	430	6 –6½	600	93	77	12.8
22	381	6½– 7	574	83	110	19.2
18	406	7 –7½	587	88	93	15.8
11	443	7½– 8	620	97	80	12.9
6	401	8 – 8½	576	88	87	15.1
10	402	8½– 9	595	88	105	17.6
2	390	9 – 9½	637	85	162	25.4
2	333	9½–10	565	72	160	28.3
2	390	10 –10½	541	85	66	12.2

* Weighted average per cent of this group is 10.7.

age, as previously determined, up or down in proportion to the amount by which the average initial record of these cows was more or less than the average initial record for all cows of the same age. For example, let us assume that a certain group of cows which were tested first as junior two-year-olds received their retests as senior three-year-olds. If the average initial

record of this group was 102 per cent of the average junior two-year-old initial records of all cows, then we may assume that if age alone were acting to increase production, when they were retested as senior three-year-olds they would still be capable of of producing 102 per cent of the average production of all cows tested initially as senior three-year-olds. Then 102 per cent of the average initial record of senior three-year-old cows would be the calculated record of this group at the age they were retested. This calculated record was compared with the actual retest records, and when these were found to be greater the difference was attributed to the development which these cows had undergone because of the handling while on previous tests.

Tables 1 and 2 set forth a detailed analysis of the two sets of Jersey and Guernsey cows with initial records as junior two-year-olds and later re-entry records. Each subgroup shows average re-entry records greater than would be expected if age alone were acting to increase the producing ability of these animals.

The study of a total of 1215 pairs of Guernsey records as outlined above showed that the average development factor for that group of animals was 12.2 per cent of the re-entry record.

Similarly, when 3722 pairs of Jersey records were studied, it was found that 11 per cent of the re-entry records of this group of animals was due to development.

The numbers are sufficiently large to make conclusions significant. The Jersey records were apportioned, according to ages at which the records were made, into 209 groups; and of this number only 23 groups, containing 39 of the total of 3722 individuals, failed to show a positive result. The Guernseys fell into 148 groups, of which only 9 groups, containing a total of 14 individuals out of the 1215, did not give a positive development factor.

Detailed results with complete tables for all groups are shown in Department Bulletin No. 1352.

THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE PERCENTAGE OF FAT IN COW'S MILK*

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The great variation in the composition of cow's milk is a matter of common observation. Studies of the causes of this variation, with regard to the fat content of milk, are numerous and have been summarized by Turner (30), who states that there are two fundamental causes of the variations. The first is the hereditary variation due to the genetic makeup of the breed and individual. The second cause appears to be due to physiological changes in the animal, caused by such factors as management, environment, lactation, reproduction and age. To the second cause Woodward (33) and Clothier (11) would add feed as a factor, and Eckles (12) adds the factor of condition of cow at time of calving. The effect of environment, or more specifically, environmental temperature, is considered in this paper.

The relation between season of the year and the per cent of fat in milk is generally known. It was shown by Eckles (13) in 1909 from a study of 240 lactation periods of cows in the Missouri and Iowa Experiment Station herds. He found that, regardless of when the lactation began, the percentage of fat when plotted followed a curve for the year, being lowest during June and July and gradually rising to the highest point in December and January, and then declining again till midsummer.

White and Judkins (32) concur in this opinion, presenting data taken on 49 cows over a period of seven and one-half years. They conclude that the milk tests lower in fat content in the

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summer months than it does in the winter months, and that this variation is due to seasonal change.

In further work done at the Missouri station, Ragsdale and Turner (28) present data on 3763 Guernsey, 299 Jersey, and 95 Holstein-Friesian, yearly records made in official testing work. Their conclusions are identical with those of Eckles. Hooper (18) alone takes exception to this belief. From a study of 88 official Jersey records, one-fourth of them beginning in each of the four seasons of the year, he concludes that "the fat percentage runs lower while the cow is fresh regardless of the time of the year she freshens," and that "there is no indication that the fat percentage runs lower in July."

In all the work mentioned thus far the season of the calendar year was the only division in the data. Eckles, however, suggested that the temperature, which of course changes with the season, might be a possible cause of the variation. Hills (17) as early as 1891 conducted two trials using the local creamery butterfat tests of the milk of 30 herds in Vermont during the months of May, June, and July, 1891, and July, August and September, 1892. He says that "The results of both series of tests point strongly to the probability that, when cows are put on pasture, the per cent of fat rises as the temperature falls, and falls as the temperature rises. In other words, the percent of fat in milk varies inversely with the temperature changes."

There is, of course, a direct relationship between the season of the year and the temperature. The knowledge that summer is a time for low tests and winter a time for high ones therefore suggests temperature as a possible cause for the variation. Clothier (11), considering the differences in feeding practices between summer and winter seasons in Arizona, finds it "impossible to believe that the seasonal variations in butterfat content of milk, observed in Arizona, are not due directly to changes in feed;" however, all other workers on this subject suggest temperature as a probable cause, and two papers have been presented to show this, where attempts were made to control other conditions.

An early piece of work is reported by Brooks (9). During December, January, February, and March, 1895, he conducted an

experiment in warming a stable for cows. Records were kept on three cows in each of two wings of his dairy barn. One wing was equipped for hot water heat and kept at a temperature of 55°F. The other wing was not artificially heated, but because of the good construction, was seldom excessively cold. He states "The most certain effect brought out by our experiments is the lowering of the per cent of fat in the milk in the warm stable."

In 1921 Ragsdale and Brody (27) reported a preliminary observation on ten cows, during the months of March and April. Their plan was to test out by direct observation the relation between the fluctuation of the environmental temperature and the fluctuation of the percentage of fat. March and April are months in which a maximum fluctuation in temperature occurs in this state. All the controllable conditions, such as feed and exercise, were kept approximately uniform throughout the period, the object being to record data showing the relation of temperature to the percentage of fat in milk, uninfluenced as far as possible under usual conditions, by other factors. From their results they conclude that there is a relation between temperature and the percentage of fat, showing roughly an increase of about 0.2 per cent in the fat for a decrease of 10°F. in the temperature, between the observed temperature limits.

The daily herd fat tests were recorded against the outdoor temperature. However, during stormy days and cool nights, the cows were kept in the barn, and while the barn was not heated artificially, it was undoubtedly warmer than the outdoor temperature. Therefore the reported temperatures were not entirely correct for the environmental temperature under which the cows were kept.

DATA ON THE UNIVERSITY OF MISSOURI DAIRY HERD UNDER CONDITIONS OF NORMAL HERD MANAGEMENT

Because of the results obtained in the preliminary study by Ragsdale and Brody (27), more quantitative data were sought, eliminating the error already mentioned in that work. Accordingly, in January, 1924, the recording of data for the entire dairy

herd of the University of Missouri was begun. Composite fat tests for all milk produced were taken daily, and a recording thermometer was used in obtaining the environmental temperature. When the cows were in the barn, the thermometer was placed in the barn; and when the cows were out of doors, the thermometer was placed out of doors. In this way the error in temperature reading, pointed out in the report of Ragsdale and Brody, was eliminated. All conditions of feeding, milking, care, and management remained normal, and the herd was handled in the usual way. Daily data for a period of 258 days was recorded. When the average daily temperatures are grouped into 10° intervals, as in table 1, the relation between the per cent of fat and the temperature is clearly shown.

TABLE 1

The daily data grouped into 10-degree intervals of temperature

NUMBER OF DAYS	AVERAGE TEMPERATURE FOR GROUP	AVERAGE PER CENT OF FAT
7	86.5	3.171
50	79.2	3.250
69	69.6	3.389
48	60.5	3.481
38	49.7	3.505
35	40.1	3.463
9	31.0	3.465
2	24.5	3.600

The gradual successive rise of the fat percentage in the temperature groups of 86.5°, 79.2°, 69.6°, 60.5°, 49.7°, and 24.5° was expected, but the drop in the 40.1° and 31° groups is unexplainable.

The 31° group is of little importance because of the small number of tests included in this average; and therefore the low average test of 3.465 per cent, while distinctly out of line with the other tests, may be somewhat discounted. However, in the 40.1° group there is a sufficiently large number of tests included, the number being 35. An explanation for this low test has not been found.

These data on the entire herd over such a long period of time,

undoubtedly contain some unavoidable errors due to the effect of some cows drying up and additional ones freshening throughout the year, but on the whole the number of cows of each breed in milk remained proportional during the entire period.

DATA ON TWO COWS UNDER CONTROLLED TEMPERATURE CONDITIONS

In the second part of the experimental work herein reported, an attempt was made to determine to what extent temperature was responsible for the variation in the fat tests, and also how much variation per unit change in temperature results.

The plan was to subject two similar cows to a constant temperature, until the percent of fat in the milk seemed at a constant level, and then to repeat the operation several times at 10° intervals of temperature, all conditions of milking and feeding remaining normal with the exception that drinking water was warmed to the experimental temperature before being offered to the cows.

The experimental animals were two mature Jersey cows as nearly alike in all respects as it was possible to get them. They were both in good flesh, and were of approximately the same nervous disposition.

For the moderate and warmer temperatures the cows were placed in a light, well ventilated room in the dairy barn, away from exciting influences. The temperature was regulated by a man in constant attendance and made more uniform by an electric fan which kept the air in motion. For the coldest temperatures the cows were placed in a large refrigerator room of a local packing house. This room was 50 by 70 by 12 feet in dimensions, and was electrically lighted. The cows were kept in a large dry compartment, but because of lack of space only one could be kept in the room at a time. It was planned to run trials at 90°, 80°, 70°, 60°, 50°, 40,° and 30°F., and to skip about from a high temperature to a low temperature in arranging the order of the trials. In this way the effect of acclimatization to any given range of temperature was eliminated. Tables 2 to 8 give the detailed numerical data on each trial.

TABLE 2

Trial I

This trial was run in the steam heated room. The planned experimental temperature was 90°F.

DATE (1924)	AVERAGE TEMPERATURE	cow 108		cow 121	
		Pounds milk	Per cent fat	Pounds milk	Per cent fat
November 1	92.0	21.1	7.204	24.0	6.420
November 2	91.7	21.5	5.750	23.5	5.927
November 3	91.9	21.7	5.284	23.3	4.811
November 4	92.3	21.7	5.078	25.1	4.845
November 5	93.5	18.3	5.085	25.9	4.979
November 6	93.7	19.1	5.513	24.6	5.344
November 7	94.8	18.3	4.685	24.7	4.831
November 8	92.3	19.9	5.155	20.5	5.211

Number days run	8
Average temperature	92.7
Average pounds milk per day per cow	20.8
Average per cent fat	5.388
Average per cent fat eliminating first day of trial	5.178

TABLE 3

Trial II

This trial was run in the steam heated room. The planned experimental temperature was 70°F.

DATE (1924)	AVERAGE TEMPERATURE	cow 108		cow 121	
		Pounds milk	Per cent fat	Pounds milk	Per cent fat
November 9	73.0	19.0	4.876	19.5	5.284
November 10	72.0	20.2	5.748	18.2	5.865
November 11	73.6	19.1	5.201	19.9	5.396
November 12	73.3	21.3	5.362	21.7	5.002
November 13	71.9	19.9	4.541	22.7	4.661
November 14	71.3	22.5	5.428	22.9	4.432

Number days run	6
Average temperature	72.5
Average pounds milk daily per cow	20.5
Average per cent fat	5.149
Average per cent fat eliminating first day of trial	5.163

TABLE 4

Trial III

This trial was run in the refrigerator room, one cow at a time. The planned experimental temperature was 40°F.

DATE (1924)	AVERAGE TEMPERATURE	cow 108		cow 121	
		Pounds milk	Per cent fat	Pounds milk	Per cent fat
November 20	44.5	17.1	5.377		
November 21	44.0	19.5	5.552		
November 22	44.0	19.4	5.756		
November 23	44.0	20.9	5.862		
November 24	43.7	19.4	5.642		
November 25	39.5	19.9	6.022		
November 26	40.5			22.0	4.790
November 27	38.0			21.0	6.550
November 28	37.5			21.5	7.046
November 29	38.0			22.0	6.390
November 30	40.5			22.0	6.529
December 1	39.0			19.0	6.992
December 2	36.5			20.0	6.780

Number days run—average of 6½ days for each cow.
Average temperature for entire period....39.9
Average pounds milk daily per cow....20.2
Average per cent fat.... 6.099
Average per cent fat eliminating first day of trial.... 6.283

TABLE 5

Trial IV

This trial was run in the steam heated room. The planned experimental temperature was 50°F.

DATE (1924)	AVERAGE TEMPERATURE	cow 108		cow 121	
		Pounds milk	Per cent fat	Pounds milk	Per cent fat
December 3	52.1	20.6	5.919	20.8	5.884
December 4	52.7	20.3	5.754	21.8	6.094
December 5	51.2	20.0	5.210	22.4	5.453
December 6	53.1	21.0	5.342	23.2	5.568
December 7	52.7	19.5	5.622	23.6	5.616

Number days run.... 5
Average temperature....52.3
Average pounds milk daily per cow....21.3
Average per cent fat....5.646
Average per cent fat eliminating first day of trial 5.582

In each of these trials it will be noted that the fat test was greatly out of line on the first day. A summary was made first using every day of each trial and another was made eliminating

TABLE 6

Trial V

This trial was run in the steam heated room. The planned experimental temperature was 60°F.

DATE (1924)	AVERAGE TEMPERATURE	cow 108		cow 121	
		Pounds milk	Per cent fat	Pounds milk	Per cent fat
December 8	59.6	20.5	5.525	22.6	5.835
December 9	60.3	19.6	5.124	20.0	5.204
December 10	61.0	19.6	5.386	22.1	5.409
December 11	62.8	20.5	5.412	23.5	5.500

Number days run	4
Average temperature	60.9
Average pounds milk daily per cow	21.0
Average per cent fat	5.424
Average per cent fat eliminating first day of trial	5.339

TABLE 7

Trial VI

This trial was run in the steam heated room. The planned experimental temperature was 80°F.

DATE (1924)	AVERAGE TEMPERATURE	cow 108		cow 121	
		Pounds milk	Per cent fat	Pounds milk	Per cent fat
December 13	79.0	19.0	5.415	23.0	5.856
December 14	80.7	19.7	5.007	23.3	5.257
December 15	80.5	20.7	4.598	23.9	5.557
December 16	79.8	19.8	5.015	23.1	5.111

Number days run	4
Average temperature	80.0
Average pounds milk daily per cow	21.6
Average per cent fat	5.227
Average per cent fat eliminating first day of trial	5.107

the first day of each trial. The comparisons were very similar in each case, however, and for this reason a summary is presented in table 9 using every day of every trial.

Throughout the running of these trials, rectal temperatures were taken and the humidity of the compartments was measured

TABLE 8

Trial VII

This trial was run in the steam heated room in very cold weather. The planned experimental temperature was 30°F.

DATE (1924)	AVERAGE TEMPERATURE	COW 108		COW 121	
		Pounds milk	Per cent fat	Pounds milk	Per cent fat
December 21	20.9	15.0	8.413	20.2	7.341
December 22	27.0	15.3	5.444	20.5	5.597
December 23	36.4	17.7	5.446	20.2	5.853
December 24	29.6	18.7	5.139	19.5	5.400
December 25	21.4	16.8	5.594	20.2	5.900

Number days run	5
Average temperature	27.0
Average pounds milk daily per cow	18.4
Average per cent fat	6.012
Average per cent fat eliminating first day of trial	5.543

TABLE 9

A summary of all of the controlled temperature trials

The trials are grouped successively by temperature changes in this table to show the comparison between fat tests.

TRIAL	TOTAL NUMBER OF DAYS	AVERAGE TEMPERATURE FOR ENTIRE TRIAL	AVERAGE POUNDS MILK PER DAY	AVERAGE PER CENT FAT FOR ENTIRE TRIAL
I	8	92.7	20.8	5.388
VI	4	80.0	21.6	5.227
II	6	72.5	20.5	5.149
V	4	60.9	21.0	5.424
IV	5	52.3	21.3	5.646
III	6½	39.9	20.2	6.099
VII	5	27.0	18.4	6.012

using a wet bulb thermometer. Body temperature of course remained normal and the relative humidity was about what would be found under normal average conditions.

DISCUSSION OF THE RESULTS

Regulation of body temperature

In seeking an explanation for the rather spectacular rise in the per cent of fat in milk, attendant upon decreasing environmental temperature, the relation between external temperature and body temperature is considered. Turner (30), in summarizing the factors which influence the per cent of fat in milk, states that variations may be brought about by physiological changes in the animal caused by environment. Lusk (22), in discussing the regulation of body temperature, points out that in cold-blooded animals body temperature varies directly with environment. The metabolism is very low at temperatures around 4°C., but increases rapidly with rising temperature. In warm blooded animals the body temperature is maintained at a constant level, independent of climatic conditions. This constant is one which is most favorable to the activity of nerve and muscle, and it is maintained in two ways:

1. *Physical regulation.* As the temperature rises the blood vessels of the skin become dilated, and heat is radiated from the skin. Evaporation of water from the body is also promoted. If the environmental temperature becomes so high that the physical regulation will not cool the body sufficiently, a supernormal temperature ensues and increased metabolism results.

2. *Chemical regulation.* In a cooling environment the contraction of the peripheral blood vessels, and the physical regulation will conserve the animal heat to a certain point. This point has been found to be 30°C. (86°F.) in guinea pigs. At this point the minimum of metabolism takes place and it is called the critical temperature. Below this point the metabolism is increased, producing more heat.

According to Barbour (1), equalization of temperature in the body is best attained through a mobile constituent such as water, which can circulate rapidly and freely. He mentions "water shifting," from blood to the brain, and vice-versa, as one of the factors in physical regulation. Osborne (26), in presenting some new aspects of the skin in temperature regulation, states that the

skin may be regarded as a gel containing water of imbibition. Evaporation of this water can occur independently of the sweat gland, and when the air is cold, dry, and in rapid motion, the evaporation may be contrary to the metabolic requirements of the body.

The effect of temperature on metabolism

In the chemical regulation of the body temperature, where more of the body tissue must be oxidized to furnish the needed heat, the metabolism of the individual is of course affected. Benedict (4) says that the basal metabolism of an individual is a function of the total mass of active protoplasmic tissue, and the stimulus to cell activity, at the time the measurement of the metabolism is taken. He mentions, as affecting basal metabolism, body weight, surface area, body composition, stimulus to cellular activity, sleep, and diurnal variation. Environmental temperature may be regarded as a stimulus to cellular activity, as was shown by Lusk (22), and it has a direct bearing upon the metabolism. Lusk quotes Rubner, who showed that extremes of temperature, both high and low, caused an increase in the basal metabolism, and that in the guinea pig 30°C. (86°F.) was the point where minimum metabolism took place.

Boothby and Sandiford (8), in a review of basal metabolism literature, state, "Experiments of Dubois show that an elevation of the temperature produces a rise in the metabolic rate of approximately 10 per cent for each degree Centigrade above the normal body temperature." Campbell and his associates (10) state that metabolism is raised by cool out-of-doors conditions, and that shivering is not necessary to raise the metabolism. McConnell and his associates (25) carried out a series of experiments with human beings to determine the correlation between metabolism and external temperature conditions. They say that, contrary to what might be expected, metabolism increases with exposure to high temperatures, Enough work has been done to substantiate this statement, but the relation remains yet to be found. Probably the difficulty in establishing this relation lies in the fact that in high temperatures the relative humidity and air movement

must be considered. They have used an "effective temperature" scale in studying the effect of heat on body metabolism. It is an index of the intensity of the heat felt by the body as a result of temperature, humidity, and air movement. Their conclusions are:

1. CO_2 produced and oxygen consumed increase with exposure to high and low temperatures.
2. Heat production increases with exposure to high and low temperatures.
3. There is a zone of minimum metabolism, between 75° and 83° effective temperatures, within which basal metabolism should be measured.
4. The metabolic rate becomes excessive when the temperature of the environment exceeds the body temperature.

The only work reviewed on the effect of temperature on the metabolism of ruminants was by Magee (24). A goat was subjected to various temperatures, from 30° to 100°F. and the metabolism rate calculated by indirect calorimetry. His conclusions were that

> The critical range of temperature for the goat in this work was from 55° to 70°F. As the temperature falls below 55°F. metabolism exhibits a slight, gradually increasing, rise, due to the oxidation necessary to cope with the increasing heat loss. As the temperature rises above 70, metabolism shows a pronounced, gradual increase, owing to the gradually increasing efforts of the animal to promote heat dissipation by panting.

The relation between the fat content of the blood and the per cent of fat in milk

A theory that the per cent of fat in the milk may have a direct relation to the fat content of the blood naturally suggests itself. It has long been known that the fat content of the blood may vary greatly at times. Bloor (7), in working with dogs, found that, with dogs in good flesh, fasting from five to seven days produced a marked increase in the fat content of the blood. He states that after the first two or three days the fasting organism depends for

its support mainly on its fat stores. The increased use of fat would tend to increase the blood fat because of the greater amount transported. He quotes Schulz as saying that this increase may be as much as 50 to 100 per cent over the blood fat in animals that are being fed regularly. This increase, however, depends upon the nutritional condition of the animals; for, while animals in good flesh show the increase, when starved, thin animals fail to show it.

Another result of starving is an increased percentage of fat in the milk produced. Eckles (12) has shown that when the ration of a cow is insufficient to support her production, she loses weight rapidly, evidently drawing upon her body fat, and the test of the milk rises. This increase in test, he states, is due to the "transfer" of fat from the body to the milk, and is most marked immediately after calving.

Starvation, then increases the fat content of the blood and also of the milk. Gage and Fish (15) supply a link in an explanation of the rise in fat content of milk with lowering temperature. They fed mammals on fat that had been stained with a fat soluble dye (sudan III) and then traced its course through the life processes of the animals. The colored fat was found in the blood, and later in the milk. In the case of the cat it required but five hours to digest, absorb, and deposit the pink fat and to turn a part of it into milk fat. With the cow, however, no pink fat could be produced in the milk, but this work at least shows that the lipoids of the blood are transported and transferred directly into the milk.

It therefore seems reasonable to assume that anything which would bring about an increase of the fat or lipoid content of the blood would effect a resultant increase in the fat content of the milk. It has been shown by Barbour (1), and Lusk (22), and others that the hemoglobin, the red blood cell count and the viscosity of the blood increase under the influence of cold environment. Under the present conceptions, such changes are attributed to the fluid concentration of the blood, and if the blood becomes more "concentrated" the percentage of each solid is

increased accordingly and the fat in the blood is therefore increased.

Low temperatures, then, cause an increase of the fat content of blood and this may result in an increased per cent of fat in the milk.

SUMMARY AND CONCLUSIONS

1. Daily data on the University of Missouri dairy herd for a period of 258 days are presented showing the average fat test and environmental temperature. The temperatures ranged from 85.5° to 24.5°F., or a net range of 62°. The average fat tests ranged from 3.171 to 3.600 per cent, or a total difference of 0.489 per cent, and it was generally found that the lower the temperature the higher the fat test. This increase in the fat test amounted to 0.079 per cent for each 10° lowering of the temperature. However, because of the many variables, this result is not considered of as much significance as the results of the controlled temperature trials.

2. Seven controlled temperature trials, on two Jersey cows, were run at 10° intervals of temperature, with all other conditions remaining normal. The range of temperature was from 92.7° to 27°F. When the seven periods were taken as a unit, there was a total temperature range of 65.7°, and a total increase in the fat test of 0.624 per cent, or an average increase of 0.095 per cent for each 10° lowering of the temperature.

3. In the controlled temperature trials there was a constant increase in the per cent of fat as the temperature dropped below 70°F. From 72.5° to 27° there was a temperature range of 45.5° and a total increase of 0.863 per cent fat, or an average increase of 0.189 per cent fat for each 10° lowering of the temperature. This is almost exactly in accord with preliminary studies at this Station by Ragsdale and Brody, who reported a rise of 0.2 per cent fat for each decrease of 10°F., between the temperature units of 70° and 30°F.

4. Above 70°F., there was an actual increase in the fat test. The reason for this is not known, but it is believed to have been due to increased metabolism, induced by higher temperature

or the result of disturbing the animals by the sudden changes from one temperature to another. The latter is indicated by the fact that the first day's test in each trial was abnormally high. When these first days were eliminated the increase in the fat test was not nearly so pronounced. It would seem that there is a range of temperature between 70° and 90°F. within which the lowest testing milk is produced. A variation in the environmental temperature either way will bring about an increase in the per cent of fat in cows milk.

5. It is believed that the controlled temperature trials are more nearly indicative of the effect of environmental temperature on the percentage of fat in cows milk than the daily herd averages where there were many variables. It is therefore concluded that all other conditions remaining constant, there is an increase of approximately 0.2 per cent fat in cows milk for each 10° lowering of the temperature within the limits of 70° and 30°F. In the second part of this experiment all conditions, other than temperature were apparently effectively controlled and remained constant throughout every change in temperature. Temperature, then, was the only variable.

6. It is concluded that temperature is a major factor in the seasonal variation of the per cent of fat in cows milk.

REFERENCES

(1) Barbour, H. G.: The heat regulating mechanism of the body. Physiol. Reviews, 1921, i, 295.

(2) Barbour, H. G.: Effects of hot and cold baths upon blood concentration and brain volume in dogs. Amer. Jour. Physiol., 1924, lxvii, 366.

(3) Barbour, H. G., and Tolstoi, E.: The rôle of the water content of the blood and its control by the central nervous system. Amer. Jour. Physiol., 1924, lxvii, 378.

(4) Benedict, F. G.: Factors affecting basal metabolism. Jour. Biol. Chem., 1915, xx, 263.

(5) Bloor, W. R.: Fat assimilation. Jour. Biol. Chem., 1916, xxiv, 447.

(6) Bloor, W. R.: Fat transport in the animal body. Physiol. Review, 1922, ii, 92.

(7) Bloor, W. R.: Variations in the fat content of the blood under approximately normal conditions. Jour. Biol. Chem., 1914, xix, 1.

(8) Boothby, W. M., and Sandiford, I.: Basal metabolism. Physiol. Reviews, 1924, iv, 69.

(9) Brooks, W. P.: Experiment in warming a stable for cows. Mass. Hatch Sta. Rep., 1895, 39.

(10) Campbell, J. A., Hargood, A., and Hill, L.: The effect of the cooling power of the atmosphere on the body metabolism. Jour. Physiol., 1921, lv, 259.

(11) Clothier, R. W.: Seasonal variations in the butterfat content of milk in Southern Arizona. Proc. Soc. Prom. Agr. Sci., 1919, xxxix, 75.

(12) Eckles, C. H.: A new factor influencing the per cent of fat in milk. Hoard's Dairyman, 1909, xl, 629.

(13) Eckles, C. H.: Jahreszeitliche Schwankugen des Prozentischen Fettgehaltes in Kuhmilch. Melchwirtschaftlickes Zentralblatt, 1909, v, 488.

(14) Erlanger, J.: Blood volume and its regulation. Physiol. Reviews, 1921, i, 177.

(15) Gage, S. H., and Fish, P. A.: Fat digestion, absorption, and assimilation in man and animals as determined by the dark field microscope and a fat soluble dye. Amer. Jour. Anat., 1924, xxxiv, 1.

(16) Gaines, W. L., and Davidson, F. A.: Relation between the percentage fat content and the yield of milk. Ill. Ag. Exp. Sta. Bull. 245, 1923.

(17) Hills, J. L.: The effect of weather upon the quality and quantity of milk. Vt. Ag. Exp. Sta. Rpt., 1892, 128.

(18) Hooper, J. J.: Studies in dairy cattle: II. Milk production. Ky. Ag. Exp. Sta. Bull. 248, 1923.

(19) Lee, F. S., and Scott, E. L.: The action of temperature and humidity on metabolism. Proc. Soc. Expt. Biol. and Med., 1914, i.

(20) Lozinsky, E.: The effects of dry and moist heat upon the body temperature and blood concentration of dogs. Amer. Jour. Physiol., 1924, lxvii, 388.

(21) Lusk, G.: The Science of Nutrition, Chap. IV, The Regulation of Temperature. Ed. 3, Philadelphia, Saunders, 1921.

(22) Lusk, G.: Influence of cold baths upon the glycogen content of man. Amer. Jour. Physiol., 1910, lxvii, 427.

(23) Magee, H. E.: The influence of variation in external temperature on the energy exchange of the goat, Jour. Agr. Sci., 1924, xiv, 507.

(24) McConnell, W. J., Yaglozlou, C. P., and Fulton, W. B.: Basal metabolism before and after exposures to high temperatures and various humidities. Public Health Reports, 1924, xxxix, 3075.

(25) Osborne, W. A.: Some new aspects of the function of the skin in temperature regulation. Jour. Physiol., 1923, lvii, p. xxvi, under proceedings.

(26) Ragsdale, A. C., and Brody, S.: The effect of temperature on the percentage of fat in milk. A first report. Jour. Dairy Sci., 1922, ii, 212.

(27) Ragsdale, A. C., and Turner, C. W.: Seasonal variations in per cent of fat in cows milk. Jour. Dairy Sci., 1922, v, 544.

(28) Riddle, O., and Honeywell, H. E.: Effects of the onset of cold weather on blood sugar and ovulation rate in pigeons. Amer. Jour. Physiol., 1924, lxvii, 337.

(29) Turner, C. W.: Factors affecting the percentage of fat in cows milk. Mo. Agr. Exp. Sta. Bull. 222, 1924.

(30) TURNER, C. W.: Seasonal variations in milk and fat production. Jour. Dairy Sci., 1923, vi, 198.

(31) WHITE, G. C., AND JUDKINS, H. F.: Variations in the fat, solids not fat, and total solids in cows' milk. Conn. Storrs Sta. Bull. 94, 1918.

(32) WOODWARD, T. E.: Factors influencing the per cent of fat in milk. Jour. Dairy Sci., 1923, vi, 466.

(33) WYLIE, C. E.: Factors influencing two-day official tests of cows. Jour. Dairy Sci., 1923, vi, 292.

SOME FACTORS AFFECTING THE GROWTH OF CERTAIN STRAINS OF P. ROQUEFORTI BLUE MOLD. II*

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The manufacturing process of Roquefort cheese and to a lesser extent that of Wensleydale cheese includes the practice of salting the curd or cheese (4) (5). The actual percentage of salt incorporated in the ripened cheese is shown in table 1.

Thom (6) shows the retarding effect of salt on the growth of thirty-one species of mold. He further demonstrates that P. roqueforti has a high salt tolerance, but the strain used was isolated from Roquefort cheese where a high percentage of salt is incorporated (6). Therefore, it is conceivably possible that wide variations in salt tolerance might exist with the different strains of P. roqueforti as isolated from Wensleydale and Roquefort cheese.

In an investigation into the manufacture of Wensleydale cheese (4) it was noted that the cheese inoculated with culture 1 (3) grew quicker and more abundantly than that inoculated with culture 16 (3). In each case the cheese contained the same percentage of salt—approximately 1 per cent (4).

OBJECT

The object of this work is to measure the rate of growth of several cultures of different strains of P. roqueforti as influenced by salt.

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[1] Thanks are tendered to the Department of Dairying of the Iowa State College, U. S. A., and to the Department of Dairying of the University of British Columbia for facilities granted and assistance given.

CULTURES USED

The cultures of P. roqueforti used were:

No. 1. P. roqueforti secured from the Dairy Division, Washington (3).
No. 16. Isolated from Wensleydale (3).
No. 32. Isolated from Wensleydale cheese inoculated with culture 16 (4).
No. 33. Isolated from Wensleydale cheese inoculated with culture I (4).

TABLE 1

AUTHORITY	REFERENCE	ORIGIN OF CHEESE	NUMBER OF ANALYSES	PER CENT MOISTURE	PER CENT ASH	PER CENT SALT
Wensleydale cheese						
Chattaway	1		1	28.30	3.7	
Golding	4	Imported cheese made by Rowntree, Yorks, England	4	28.76	1.74	0.12
Golding	4	Made at University of British Columbia	8	24.82	3.21	0.99
Roquefort cheese						
Matheson	5	Roquefort from France	?	38.0	6.0	4.0
Golding	4	Roquefort from cow's milk. Made at University of British Columbia	?	36.0	4.77	3.33

MEDIA

Milk. Sweet skim milk in which no bacterial change had taken place was used in every case. Test tubes of uniform diameter were selected, filled with exactly 10 cc. of milk, plugged and sterilized.

Milk + salt. A good grade dairy salt of 99 per cent purity was used. The dried salt was weighed into sufficient dry test tubes in amounts of 0.4, 0.8, 1.2, and 1.6 grams per tube. These tubes were plugged and dry sterilized. When both the 10 cc. tubes of milk and salt were cool, the salt was added to the milk

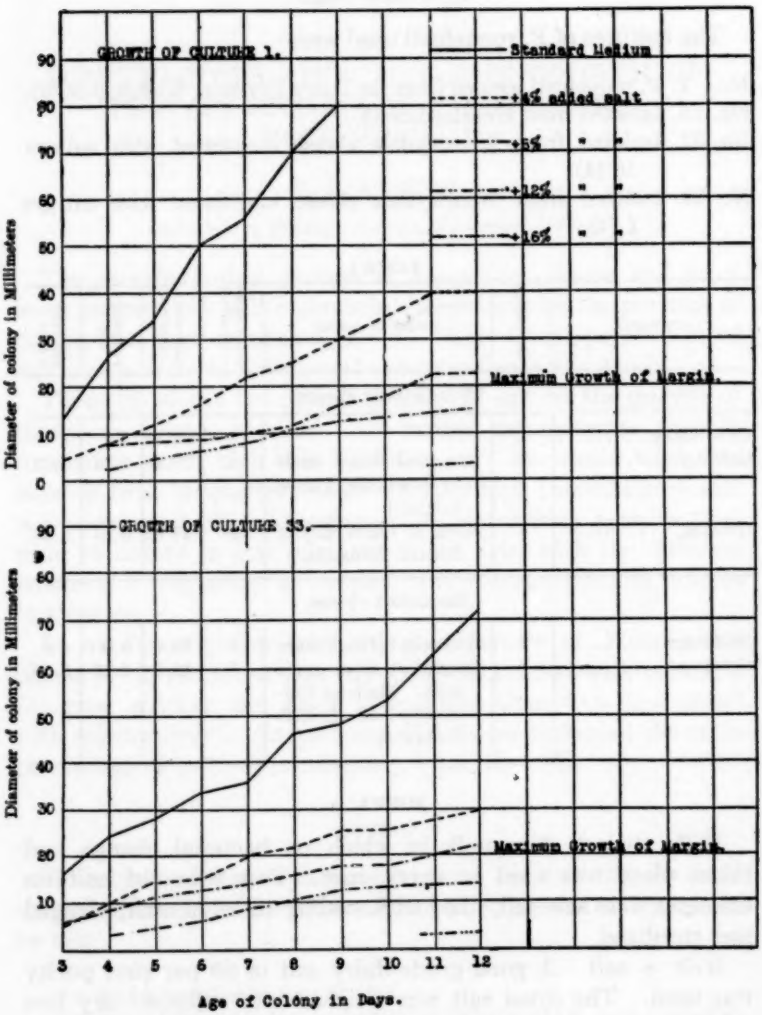
GROWTH OF CULTURE 1.
Standard Helium
+4% added salt
+8% " "
+12% " "
+16% " "
Maximum Growth of Margin.
Diameter of colony in Millimeters
GROWTH OF CULTURE 33.
Maximum Growth of Margin.
Diameter of colony in Millimeters
Age of Colony in Days.

FIG. 1

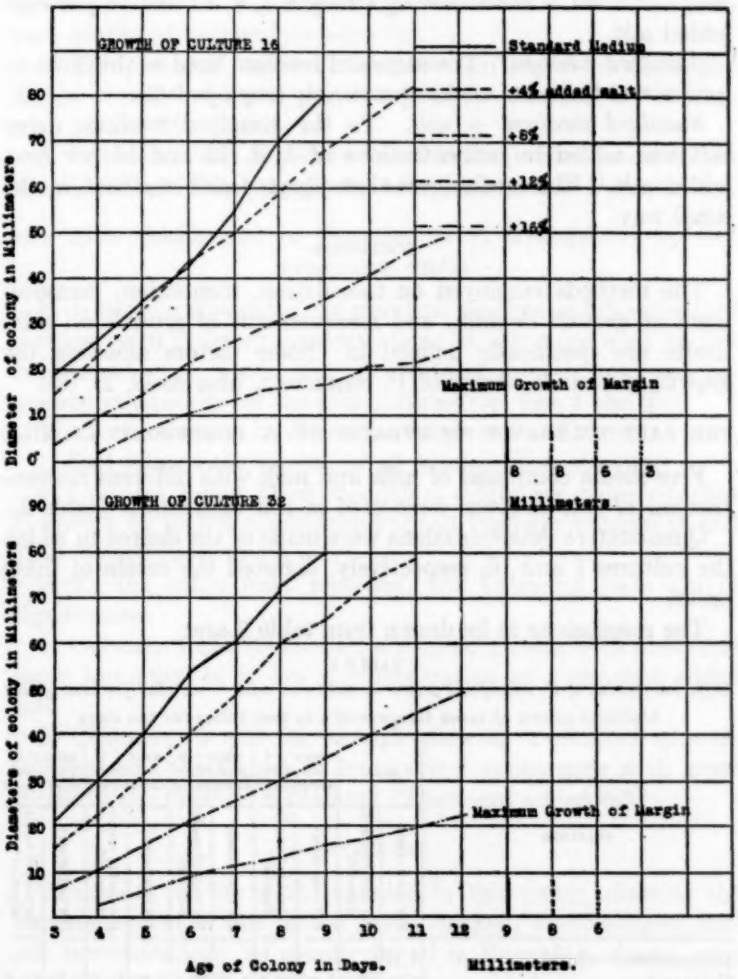
GROWTH OF CULTURE 16
Standard Medium
+4% added salt
+8%
+12%
+16%
Maximum Growth of Margin
Diameter of colony in Millimeters
8 8 6 3
Millimeters
GROWTH OF CULTURE 32
Diameter of colony in Millimeters
Maximum Growth of Margin
3 4 5 6 7 8 9 10 11 12 9 8 6
Age of Colony in Days.
Millimeters.

FIG. 2

under sterile conditions. Thus concentrations of salt and milk were obtained of the following strength 4, 8, 12 and 16 per cent added salt.

Standard medium. The standard medium used in this investigation was the same as that previously employed (2).

Standard medium + salt. To the standard medium dairy salt was added in concentrations of 4, 8, 12 and 16 per cent added salt. The media were then plugged and sterilized in the usual way.

METHODS

The methods employed on inoculation, incubation, measurement of growth in milk, and measurement of growth on solid media are specifically defined in "Some factors affecting the growth of certain strains of P. roqueforti, blue mold, I" (2).

THE SALT TOLERANCE OF STRAINS OF P. ROQUEFORTI IN MILK

Five media composed of milk and milk with different concentrations of salt as given were used in this experiment (table 2).

Quantitative determinations were made of the degree to which the cultures 1 and 16, respectively, digested the casein of these milks.

The conclusions to be drawn from table 2 are:

TABLE 2

Different strains of P. roqueforti grown in milk and milk + varying per cent of salt

Cultures grown at room temperature in test tubes for ten days

CULTURES	MILK		MILK + 4 PER CENT ADDED SALT		MILK + 8 PER CENT ADDED SALT		MILK + 12 PER CENT ADDED SALT		MILK + 16 PER CENT ADDED SALT	
	Per cent casein	Per cent of casein digested*	Per cent casein	Per cent of casein digested*	Per cent casein	Per cent of casein digested*	Per cent casein	Per cent of casein digested*	Per cent casein	Per cent of casein digested*
Uninoculated control	3.10									
Culture 1	2.20	29.0	2.27	26.8	2.50	19.4	2.76	11.0	2.76	11.0
Culture 16	2.79	10.0	2.91	6.1	2.97	4.2	2.97	4.2	2.95	4.8

* This figure represents the percentage of the total casein that has been rendered soluble.

1. Culture 1 (Roquefort origin) has a greater power to digest casein in milk and salted milk than has culture 16. This is in part confirmed by previous work (3).

2. The power of culture 1 to grow and digest casein in the higher concentrations of salt is relatively less than is the case with culture 16.

3. Concentrations of salt as found in Roquefort cheese would retard but not inhibit the growth of either strain of P. roqueforti.

THE SALT TOLERANCE OF STRAINS OF P. ROQUEFORTI IN THE STANDARD MEDIA

Five media composed of the standard medium and the standard medium with different concentrations of salt were used in this experiment (figs. 1 and 2). The rate of growth has been expressed graphically for the four cultures (see figs. 1 and 2).

The conclusions to be drawn from figures 1 and 2 are:

1. Salt retards the growth of all four cultures of mold.

2. Cultures 1 and 33 of Roquefort origin are more retarded in growth by high concentrations of salt than are cultures 16 and 32 of Wensleydale origin.

From the other data recorded, the following facts are of significance:

1. Cultures 1 and 33 showed a stunted growth with abnormal spore formation in as low a concentration as 4 per cent added salt.

2. Cultures 16 and 32, though showing a stunted growth, still retained their more or less typical appearance with green spores in the medium containing 12 per cent added salt.

SUMMARY

The result of the work recorded in this paper, showing the retarding effect of salt on the media defined, would suggest that salt concentrations, as usually found in Roquefort cheese, may retard but not inhibit the growth of the mold of either strain.

With Wensleydale cheese, where the percentage of salt in the cheese is lower, the salt would not be expected to be an important inhibiting factor.

The failure of culture 16 to grow as well as culture 1 in Wensleydale cheese must be accounted for by other factors than salt.

REFERENCES

(1) DOANE, C. F., AND LAWSON, H. W. 1918 Varieties of cheese; descriptions and analysis. Bull. 608, U. S. Dept. of Agr., Washington.

(2) GOLDING, N. S. 1926 Some factors affecting the growth of certain strains of *P. roqueforti* blue mold. I. Jour. Dairy Sci., ix, no. 1.

(3) GOLDING, N. S. 1925 The mold associated with the ripening of blue veined cheese. Mycologia, xvii, no. 1, Lancaster.

(4) GOLDING, N. S. 1924 Wensleydale cheese. Part I. Manufacture on the coast of British Columbia. Part II. Mycological and chemical studies. In manuscript.

(5) MATHESON, K. J. 1921 Manufacture of cow's milk Roquefort cheese. Bull. 970, Bur. An. Ind. U. S. Dept. of Agr., Washington.

(6) THOM, C. 1914 The salt factor in the mold-ripened cheeses. Bull. 79, pp. 387–394. Storrs Agr. Exp. Sta., Conn.

(7) WALKER-TISDALE, C. W. 1922 Wensleydale cheese—how to make both types. Milk Industry, ii, no. 8.

AN ALGEBRAIC METHOD OF PROPORTIONING ICE CREAM MIXES*

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This discussion explains a method of proportioning and standardizing ice cream mixes which has been presented to students in the Department of Dairy Industry at Cornell University each year since 1921. The flexibility of its application to any mix requirements and the simplicity of the calculations have received the approval of all classes of students interested in ice cream making. Continued use of the method under commercial conditions where speed and accuracy are essential has demonstrated its usefulness.

Materials which are used in making ice cream may be divided into two classes; milk products and those products which are not derived from milk. The milk products furnish, generally speaking, the milk fat, milk solids not fat (msnf), and the bulk of the water in the mix. Trade demands and marketing conditions require the presence of definite amounts of sugar, gelatin, flavors, etc., so that it is by correctly proportioning the amounts of milk products that the desired amount of fat and milk solids not fat are obtained in the ice cream.

To simplify the explanation of this method of standardizing, the solution of a typical problem will be demonstrated.

Under conditions which are assumed, it is necessary to make 1000 pounds of ice cream mix containing 10 per cent fat, 11 per cent msnf, 13.5 per cent sugar, and 0.5 per cent gelatin dissolved in 6 per cent water.

* Received for publication November 16, 1925.

The available materials and their composition are: Any amount of:

	FAT	MSNF	SUGAR	GELATIN
	per cent	*per cent*	*per cent*	*per cent*
Cream	40	5.4		
Whole milk	4	8.8		
Skim condensed	0.1	27.0		
Sugar			100	
Gelatin				100

The first step of the solution consists in determining the amounts of the materials not milk which must be used in the mix.

1000 pounds mix × 0.135 = 135 pounds sugar
1000 pounds mix × 0.005 = 5 pounds gelatin } gelatin solution
1000 pounds mix × 0.06 = 60 pounds water }
Total weight of non-milk products = 200 pounds

The difference between the total weight of the mix desired, 1000 pounds, and the weight of the ingredients other than milk in the mix, 200 pounds, indicates that the cream, whole milk, and condensed milk must furnish a total of 800 pounds.

It is evident that the difference between the total weight of the milk products in the mix and the combined weights of cream and condensed milk, will equal the weight of the whole milk. Stated as an equation this fact appears as:

800 − pounds of cream − pounds of condensed milk = pounds of whole milk

If pounds of cream = x and pounds of condensed milk = y, then

$$800 - x - y = \text{pounds of milk}$$

Using the above symbols again, it is possible to state that:

The pounds of fat derived from the cream = 40 per cent of x.
The pounds of fat derived from the condensed = 0.1 per cent of y.
The pounds of fat derived from the milk = 4 per cent of $(800 - x - y)$.

The desired mix must contain 10 per cent fat, or 1000 times

0.10, which is 100 pounds of fat. Since the above milk products furnish all the fat in the mix, the Fat Equation can be formed.

$$\text{Fat equation } 0.40x + 0.001y + 0.04\,(800 - x - y) = 100$$

It is possible by the same reasoning to state that:

The pounds of milk solids not fat derived from the cream = 5.4 per cent of x
The pounds of milk solids not fat derived from the condensed = 27 per cent of y
The pounds of milk solids not fat derived from the milk = 8.8 per cent of $(800 - x - y)$.

The desired mix must contain 11 per cent milk solids not fat, or:

1000 times 0.11 = 110 pounds of Msnf

Since the milk products furnish all the msnf, these facts may be used to form the msnf equation.

$$\text{Msnf equation } 0.054x + 0.27y + 0.088\,(800 - x - y) = 110$$

The solution of these equations may be arrived at through methods known to any one acquainted with elementary algebra. A simple procedure is indicated here.

The cumbersome decimals of these equations may profitably be eliminated by multiplying the numbers of each equation by 100.

$$\text{Fat equation } 40x + 0.1y + 4\,(800 - x - y) = 10{,}000$$
$$\text{Msnf equation } 5.4x + 27y + 8.8\,(800 - x - y) = 11{,}000$$

Simplifying and combining:

$$\text{Fat equation } 36x - 3.9y = 6800$$
$$\text{Msnf equation } -3.4x + 18.2y = 3960$$

One of the unknown quantities must be eliminated. To accomplish this multiply each number of the fat equation by the first number of the msnf equation,

$$36x - 3.9y = 6800$$
$$3.4$$

$$\text{Fat equation } 122.4x - 13.26y = 23{,}120$$

Then multiply each number of the msnf equation by the first number of the fat equation.

$$-3.4x + 18.2y = 3960$$
$$36$$

$$\text{Msnf equation } -122.4x + 655.2y = 142{,}560$$

Adding the fat and msnf equations, the values for x cancel each other,

$$\begin{aligned} \text{Fat equation} \quad & 122.4x - 13.26y = 23{,}120 \\ \text{Msnf equation} \quad & -122.4x + 655.2y = 142{,}560 \end{aligned}$$

$$641.94y = 165{,}680$$
$$y = 258.092 \text{ pounds of condensed milk}$$

Substituting this value for y in

$$\text{Fat equation } 36x - 3.9y = 6800, \text{ then}$$
$$\text{Fat equation } 36x - 3.9 \times 258.092 = 6800$$

Combining and transposing,

$$36x = 7806.55$$
$$x = 216.848 \text{ pounds of cream}$$

Since

$$800 - x - y = \text{pounds of milk}$$

therefore

$$800 - 216.848 - 258.092 = 325.06 \text{ pounds of milk}$$

Formula for desired mix

	MATERIALS	FAT	MSNF	SUGAR	GELATIN	WATER
	pounds	*pounds*	*pounds*	*pounds*	*pounds*	*pounds*
Cream	216.848	86.7392	11.7097			
Whole milk	325.06	13.0024	28.6052			
Condensed milk	258.092	0.2580	69.6848			
Sugar	135			135		
Gelatin	5				5	
Water	60					60
Total	10000	99.9996	109.9997			
Desired	10000	100	110	135	5	60

It may be necessary to incorporate certain odd lots of milk, cream, or condensed milk into a mix in addition to the regular ingredients. To accomplish this, determine the pounds of fat, milk solids not fat and sugar furnished by each small lot of product to be used and deduct these amounts from the amount of fat, milk solids not fat, and sugar required by the mix. Deduct the total weight of each lot of milk, cream, or condensed from the calculated weight of milk products required by the mix. The mix can then be proportioned by using the proper amounts of the milk products which must furnish the remaining amounts of fat and milk solids not fat of the mix. The amounts of these milk products can be calculated by the use of the equations whose derivation has already been explained.

Sometimes sweetened condensed milk must be used in making ice cream. Under such conditions the condensed will furnish part of the sweetening of the mix. Two methods of calculation may be used under such circumstances. In the first place the mix may be calculated in exactly the manner explained in the original problem by disregarding temporarily the sugar content of the condensed. When the amount of each milk product has been determined then the pounds of sugar in the condensed should be deducted from the calculated amount of cane sugar necessary. The difference equals the pounds of sugar which must be added to the mix. As an example, it may be assumed that a certain set of conditions requires the addition of 300 pounds of condensed milk containing 120 pounds of sugar to a mix which needs 400 pounds of sugar for sweetening. In this case it is necessary to add 280 pounds of sugar. This obviously leaves the mix 120 pounds short of the correct weight, and this difference in weight must be corrected by the addition of water.

When it is undesirable to add water to a mix and sweetened condensed must be used, the procedure must be varied slightly. Assume, for example, that 100 pounds of sweetened condensed contains 40 per cent sugar, 6 per cent fat, and 30 per cent msnf. If the sugar could be removed, there would remain 60 pounds of a theoretical condensed milk containing 10 per cent fat and

50 per cent msnf. The percentage composition of the theoretical condensed milk can then be used in calculating the necessary amounts of milk and cream by means of the equations which have been explained. The amount of theoretical condensed milk represents only 60 per cent of the real condensed milk. Therefore, the weight of this theoretical condensed milk calculated as necessary for the mix must be multiplied by 100/60 to obtain the true amount of condensed milk to be used in the mix. The difference between the theoretical and the real weight of the condensed milk equals the weight of the sugar which must be deducted from the total amount required to sweeten the mix.

When the mix ingredients are placed in a mixing tank, it is common practice to determine the composition of the whole mix. It may happen that it does not conform to the desired percentage composition. This inaccuracy may be due to one or both of two errors; inaccurate analysis of the ingredients which were used in calculating the formula, or inaccurate weighing of the ingredients at the time of mixing. When corrections are necessary, the same procedure may be used which was explained in connection with the use of sweetened condensed milk. The mix must be combined with the products which will correct its inaccuracy. In forming the equations, it is best to use the percentage composition of a theoretically non-milk-product free mix. For example a mix which is composed of 10 per cent fat, 11 per cent msnf, 14.5 per cent sugar, and 0.5 per cent gelatin contains 15 per cent or 15 pounds per hundred of non-milk products. The weight of theoretical non-milk-product free mix, therefore, equals 85 pounds. The percentage composition of this theoretical non-milk-product free mix may be expressed as $\frac{10}{85} \times 100$ or 11.7 per cent fat and $\frac{11}{85} \times 100$ or 12.9 per cent msnf. The remaining calculations are identical with the procedure of proportioning a mix in which a theoretically sugar free condensed milk is used as one of the ingredients.

Some workers prefer to use formulas in the proportioning of a mix. Under such circumstances the following expressions for the amounts of necessary materials may be found useful. These

expressions are solutions of the equations already described in which letters are used in place of numbers.

x = pounds of cream or butter, etc.
y = pounds of condensed or powdered milk, etc.
m = pounds of milk products in the mix
$m - x - y$ = pounds of milk or skimmilk, etc.
a = per cent of fat in cream, etc.
b = per cent of fat in condensed milk, etc.
c = per cent of fat in milk, etc.
d = per cent of msnf in cream, etc.
e = per cent of msnf in condensed milk, etc.
f = per cent of msnf in milk, etc.
F = pounds of fat desired in the mix
S = pounds of msnf desired in the mix

$$x = \frac{(F - cm)(e - f) - (S - fm)(b - c)}{(a - c)(e - f) - (d - f)(b - c)} = \text{pounds of cream, etc.}$$

$$y = \frac{(a - c)(S - fm) - (d - f)(F - cm)}{(a - c)(e - f) - (d - f)(b - c)} = \text{pounds of condensed milk, etc.}$$

$$m - x - y = \text{pounds of milk, etc.}$$

The application of these formulas may be illustrated by the problem which has been solved in this discussion by substituting in each formula the proper values of the letters.

Substituting in the expression for the pounds of cream:

$$x = \frac{[100 - (0.04 \times 800)][0.27 - 0.088] - [110 - (0.88 \times 800)][0.001 - 0.04]}{(0.40 - 0.04)(0.27 - 0.088) - (0.054 - 0.088)(0.001 - 0.04)}$$

Simplifying:

$$x = \frac{[(100 - 32) \times 0.182] - [(110 - 70.4) \times (- 0.039)]}{(0.36 \times 0.182) - (- 0.034 \times - 0.039)}$$

Simplifying:

$$x = \frac{12.376 + 1.5444}{0.06552 - 0.001326} = \frac{13.9204}{0.06419} = 216.84 \text{ pounds of cream}$$

It may be noted that the denominator in the formula for the value of y is identical with the denominator in the expression

for the value of x. The equation for the value of y can then be written as:

$$y = \frac{(a - c)(S - fm) - (d - f)(F - cm)}{0.06419}$$

Substituting:

$$y = \frac{[0.40 - 0.04][110 - (0.088) \times 800)] - [0.054 - 0.088][100 - (0.04 \times 800)]}{0.06419}$$

Simplifying:

$$y = \frac{[0.36 \times (110 - 70.4)] - [-0.034 \times (100 - 32)]}{0.06419}$$

Thus:

$$y = \frac{(0.36 \times 39.6) - (-0.034 \times 68)}{0.06419} = \frac{14.256 + 2.312}{0.06419}$$

$$y = \frac{16.568}{0.06419} = 258.09 \text{ pounds of condensed milk}$$

$$m - x - y = \text{pounds of milk}$$

Substituting:

$$800 - 216.84 - 258.09 = 325.07 \text{ pounds of milk}$$

One difficulty may be encountered in proportioning or standardizing mixes by this or any other method. The operator may select materials whose composition make a desired combination impossible. Under such circumstances the value for x or y may be a minus quantity or their sum may be greater than the total amount of milk products possible in the mix.

REVIEW OF FOREIGN DAIRY LITERATURE

J. L. HILEMAN

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VIRTANEN. *On the Decomposition of Casein by Bact. casei and Strep. lactis.* (Societas Scientiarum Fennica Commentationes physico-mathematicae, Vol. 1, No. 41, p. 13, March, 1923. Abstracted in Le Lait, Vol. 5, August–September, 1925, p. 756.)

The author shows that Bact. casei ϵ, which cannot decompose casein at room temperature under ordinary conditions, can do so at 20° if the cultures have previously been maintained at 40° for twenty-four hours, which approximates the conditions of manufacture of Emmenthal cheese. He inoculated the organism into a half-liter of skim milk and added 30 grams of calcium carbonate. These cultures were maintained at 18° for one to two months, and were then analyzed for soluble nitrogen, amine nitrogen and ammoniacal nitrogen.

The Bact. casei ϵ died rapidly in the milk cultures containing the calcium carbonate, just as they do in cheese. However, Virtanen holds that they are still responsible for the decomposition of casein that takes place, this decomposition being due to an enzyme secreted by the organisms which continues to act after their death. From this the author argues that Bact. casei ϵ is the agent in the ripening of Emmenthal cheese, even though this cheese contains no Bact. casei ϵ after four to six months, but only Bact. casei α.

Strep. lactis, under the conditions found in the manufacture of Emmenthal cheese, also attacks casein. This organism and Bact. casei ϵ break down casein more vigorously when both are present than when only one is present, due to some symbiotic action.

GYORGY. *On the Distribution of Calcium and Inorganic Phosphorus in Milk.* (Biochemische Zeitschrift, Vol. 142, Nos. 1 and 2, October, 1923. Abstracted in Le Lait, Vol. 4, November, 1924, p. 779.)

This investigator attempts to answer the question as to whether or not there is a close relationship between the non-diffusible calcium phosphate and the casein in milk. He shows that the inorganic phosphates pass entirely into the serum obtained when milk is allowed to sour naturally, while only 40 per cent are found in the serum obtained when ren-

net acts on milk. That part of the phosphorus which will not pass through a Haen ultra-filter is 50 to 60 per cent in cow's milk, and 30 to 40 per cent in human milk.

At the iso-electric point of casein, all of the calcium and phosphorus are found dissolved in the serum. A solution of casein treated with lime and phosphoric acid gives the same results as milk.

These results are in close agreement with the work of Van Slyke and Bosworth at Geneva.

The author draws the conclusion that there is a close chemical relationship between the casein and the non-diffusible calcium phosphate of milk.

BARTHEL. *Influence of Molds on Lactic Ferments.* (Le Lait, Vol. 4, November, 1924, p. 725.)

Bacillus subtilis increases the activity of cultures of Streptococcus lactis. Certain molds also have such a symbiotic effect, among them being Mucor piriformis, Aspergillus niger and Penicillium glaucum. Oidium lactis appeared to have no effect.

CHARLES VAS. *Formation of Acid in Symbiosis by Bacillus bulgaricus and Streptocccus lactis in Yoghourt.* (Le Lait, Vol. 4, October, 1924, p. 625.)

Yoghourt is made by incubating milk containing a culture of Bacillus bulgaricus and Streptococcus lactis at 45°C. till coagulation occurs, and then reducing the temperature to 18° to 20°C. Under these conditions the mixed culture produces more acid, and produces it more quickly, than is produced by either organism is pure culture.

H. ROEDER. *Study of the Progress of Rennet Coagulation of Milk.* (Schweizerische Milchzeitung, No. 54, June, 1923. Abstracted in Le Lait, Vol. 4, November, 1924, p. 780.)

Roeder describes a unique apparatus for studying the rennet coagulation of milk, consisting of a piece of black glass moved by clockwork in such a way that it comes from the interior of the body of milk to the surface twice every second. The milk is placed in a vessel in a constant-temperature bath. The rennet is placed in a small boat floating on the surface of the liquid, and this is capsized by means of the thermometer used to observe the temperature of the milk at the required instant. The black glass coming periodically to the surface makes it possible to observe easily the appearance of the first floccules of curd, and to follow the course of the coagulation closely to completion.